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14. Abstract
We used systematic multiplex rt-pcr and dna microarray hybridization methods to examine the expression of the genes in the chromosomal regions of 18q21-qter, 8p, and 1p33-pter, which are often decreased in copy number in breast tumors and cell lines derived from breast cancer. We identified dozens of genes in the chromosomal regions whose expression was frequently diminished or lost in breast cancer cell lines that were examined. We confirmed the results by real-time qrt-pcr. We also examined the expression of those genes in the clinical specimens of breast cancer and observed the down-regulation in expression of some of them in the clinical specimens of breast cancer. Those genes included ccbe1, tcf4, np_115536.1, and np_689683.2 in 18q21-qter, and myom2, np_859074, np_001034551, nrg1, phyip (phyhip), q7z2r7, sfrp1, and sox7 in 8p.

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INTRODUCTION

Normal breast epithelial cells undergo both genetic and epigenetic changes in their malignant progression to cancer (1). In that progression, changes in proto-oncogenes and tumor suppressor genes play an important role. Because the (sub-) chromosomal loss (or decrease) of tumor suppressor genes and the gain (or amplification) of proto-oncogenes may confer cells with growth advantages, these changes provide an important tool to identify genes that are important in carcinogenesis. Several consistent changes have been reported, from karyotyping analyses of breast tumors and cancer cell lines (2, 3). By scanning the changes in gene expression and DNA methylation, we proposed to identify tumor suppressor genes in the chromosomal regions of 1p33-pter, 8p, and 18q, three of the regions that are most consistently decreased in copy number.

BODY

The following four tasks and timelines were proposed:

Task 1. To construct oligodeoxynucleotide microarrays representing *NotI* sites in the chromosomal regions of 1p33-pter, 8p, and 18q (months 1–12):

- Identify all the *NotI* sites in chromosomal regions 1p33-pter, 8p, and 18q followed by determining the size of the *NotI-MseI* DNA fragments;
- Select, out of roughly 800 *NotI* sites in those regions, more than 500 gene promoter-associated *NotI* sites that are located within 2 kbp from the nearest *MseI* sites;
- Design ~50–60mer oligodeoxynucleotides representing those *NotI-MseI* DNA fragments; and
- Prepare oligodeoxynucleotide microarrays.

Task 2. To identify tumor suppressor gene candidates by DNA microarray MS-AFLP (months 9–30):

- Perform the DNA microarray MS-AFLP hybridization experiments using genomic DNA from normal breast epithelial cells and three breast cancer cell lines (MCF7, BT-20, and MDA-MB468); and
- Identify the *NotI* sites that exhibit a decrease in spot intensity in the cells of those three breast cancer cell lines, followed by the examination of neighboring genes.

Task 3. To examine gene expression by SM RT-PCR (months 19–36):

- Establish the SM RT-PCR system composed of ~10 candidate genes located in the 3 chromosomal regions indicated in Task 1;
- Perform SM RT-PCR experiments using RNA from normal breast epithelial cells and three breast carcinoma cell lines (MCF7, BT-20, and MDA-MB468); and
- Identify genes that are down-regulated in expression in those breast cancer cell lines.

Task 4. To examine homozygous deletion by SM PCR (months 19–36):

- Perform the SM PCR experiments using genomic DNA from normal breast epithelial cells and the same three breast carcinoma cell line cells; and
- Identify genes that are homozygously deleted in those breast cancer cell lines.

In Year 1, we planned to perform Task 1 and part of Task 2. We started with Task 1 and identified all the *NotI* sites in the chromosomal regions of 1p33-pter, 8p, and 18q for the *NotI-MseI* MS-AFLP analysis (4, 5). We soon obtained preliminary results indicating that the proposed detection method of DNA methylation alterations would allow the coverage of a lesser

number of genes than we expected. This was because *NotI* sites tended to cluster rather than spread evenly over the genome. For example, the chromosomal region of 18q contains 411 genes and 174 *NotI* sites. If one *NotI* site is present per gene, more than 40% of the genes will be examined for changes in DNA methylation. However, fewer than 80 genes were demonstrated to actually possess *NotI* sites in their promoter regions. This amounts to only 20% of the genes in that region. Assuming that the sensitivity of the *NotI-MseI* DNA microarray MS-AFLP method is 75% using oligonucleotide probes as we previously determined, we can analyze only 60 out of the 411 genes. Additionally, the methylation status of the promoter region of one gene may not necessarily characterize the methylation statuses of its neighboring genes or coincide reciprocally with the transcription of the gene. Therefore, we directly proceeded to Tasks 3 and 4 to identify the genes that exhibit differences in expression and copy number between the primary culture of normal mammary epithelial cells and established breast cancer cell lines.

We examined the expression of 127 genes in the chromosomal region of 18q21-qter in normal and cancerous breast cells and tissues. Rather than analyze the entire region of 18q, we analyzed the genes in the chromosomal region of 18q21-qter. By focusing our efforts on the genes located in cytobands where potential tumor suppressor genes are likely be located (6), the total number of protein-coding genes for analysis was reduced from 223 to 140. We used two different techniques to examine gene expression: systematic multiplex RT-PCR (SM RT-PCR) and DNA microarray hybridization. We used the Illumina BeadChips for DNA microarray hybridization. We identified several interesting genes that exhibited differences in gene expression. Partial or entire loss of expression was observed in genes such as CCBE1, CCDC11, CD226, NP_115536.1, NP_689683.2, RNF152, SERPINB8, and TCF4 in a majority of breast cancer cell lines that were examined. An increase in gene expression was rare, but found with the transcription factor ONECUT2 gene in all the cancer cell lines. We further examined the expression of the selected genes from 18q21-qter by *real-time* qRT-PCR. We did this not only with the cDNA specimens from breast cancer cell lines that were previously used in the SM RT-PCR studies, but also with the cDNA specimens prepared from the matched pairs of normal and cancerous breast tissues from breast cancer patients. *Real-time* qRT-PCR experiments confirmed that the SM RT-PCR results obtained with the breast cancer cell lines were correct. Analysis of clinical specimens of breast cancer demonstrated that the gene expression of CCBE1, TCF4, NP_115536.1, and NP_689683.2 was down-regulated in the majority of clinical cases of breast cancer. We also performed copy number analysis by SM PCR and also by arrayCGH. We found homozygous deletions of the SMAD4 and ELAC1 genes in the MDA-MB468 breast cancer cell line.

In Year 2, we examined the expression of 273 genes located on the p-arm of chromosome 8 in breast cancer cell lines by SM RT-PCR and DNA microarray hybridization using the Illumina BeadChips. We observed frequent decreases in expression of approximately two-dozen genes and increases in expression of several genes on this chromosomal arm. These changes in gene expression of the cell lines were later confirmed by *real-time* qRT-PCR. Additionally and more importantly, we found that a number of these variations were also observed in the majority of clinical cases of breast cancer that we examined. These included down-regulation of the MYOM2, NP_859074, NP_001034551, NRG1, PHYIP (PHYHIP), Q7Z2R7, SFRP1, and SOX7 genes and up-regulation of the ESCO2, NP_115712 (GINS4), Q6P464, and TOPK (PBK) genes. We did not observe any genes that were homozygously deleted in the breast cancer cell lines examined by SM PCR and arrayCGH.

In Year 3, we examined the expression of 624 genes in the chromosomal region of 1p33-pter by DNA microarray hybridization using Illumina BeadChips. We also analyzed the

expression of some of the genes by SM RT-PCR. As opposed to the chromosomal regions of 18q21-qter and 8p, we did not find particularly interesting candidate genes that exhibited down-regulation in expression in the 1p33-pter region. Although there were genes that exhibited decreased expression in several cancer cell lines in comparison with primary cultures of normal epithelial cells, the frequency was low for most of the genes. The PLCH2 gene was down-regulated in a majority of breast cancer cell lines that were analyzed. However, the expression in breast tumor tissues increased. A few other genes, such as HES5 and AJAP1, seem to be down-regulated in a majority of cell lines and await further examination by *real-time* qRT-PCR for confirmation.

Rather than determining the methylation statuses of the promoter regions of the candidate genes with potential tumor suppressor activity, we proceeded to construct the cDNA expression constructs of many of these candidate genes in a eukaryotic expression vector, pcDNA3.1/V5-His. This decision was based on our belief that gene expression is more important than methylation of the promoter for gene functionality.

KEY RESEARCH ACCOMPLISHMENTS

We have just finished the third year of the 3-year project. The key research accomplishment for the entire period of research is the determination of changes in expression of the genes in the chromosomal regions of 18q21-qter, 8p, and 1p33-pter. We did the analysis not only by the DNA microarray hybridization method, but also by the SM RT-PCR method. This use of these two methods complemented each other. The DNA microarray hybridization allows quantitative measurement for moderately to highly expressed genes but often fails to detect weakly expressed genes. The SM RT-PCR method allows semi-quantitative detection of weakly expressed genes. Our study has identified more than a dozen genes that are down-regulated in gene expression in breast cancer cell lines and also in clinical specimens of breast cancer in the chromosomal regions. We have constructed eukaryotic expression constructs of these genes, and they are waiting to be tested for functionality in tumor suppression activity.

REPORTABLE OUTCOMES

We have already published the results obtained from the copy number and expression analysis of the genes in the chromosomal region of 18q21-qter (7). The PDF of the paper is included in the Appendices. We have submitted a manuscript describing the results from the copy number and expression analysis of the genes in the chromosomal arm of 8p and are waiting for the outcome of the peer review. The PDF of the manuscript is also included in the Appendices. We have not yet finished the SM RT-PCR analysis of many of the genes in the chromosomal region of 1p33-pter. Although the DNA microarray hybridization experiments of all the genes in the region did not identify any particularly intriguing candidate genes, SM RT-PCR may identify several genes that are down-regulated in breast cancer among the genes that are weakly expressed. Once the work is completed, we will publish the results. Additional funding will be necessary to perform DNA transfection experiments of the eukaryotic expression constructs of those candidate genes that have been identified in this study, and we are trying to obtain the funding to secure the continuation of this important research.

CONCLUSIONS

We have learned several important lessons from the completed research. First, methylation status of gene promoter does not always coincide with gene transcription activity. The genes with

hypomethylated promoter are occasionally unexpressed. On the other hand, the genes with hypermethylated promoter tend to be unexpressed as anticipated. Second, differences in gene expression between the primary cultures of normal breast epithelial cells and established breast cancer cell lines are often reproduced in the clinical specimens of normal and cancerous pairs of breast tissues. However, there are cases where the differences were reversed in the clinical specimens. The most prominent case was found with the PLCH2 gene, where the expression is down-regulated in all the breast cancer cell lines examined, but up-regulated in a majority of tumors. One possible explanation is that the changes in the cells other than epithelial cells in cancerous tissues may be responsible. Because this phenomenon may implicate an important interaction between breast cancer cells and normal cells surrounding them in tissue, we would like to determine the cause of this discrepancy and report it in future results.

The expression analysis of the genes in the chromosomal regions that are frequently deleted in breast cancer resulted in the identification of candidate genes with potential tumor suppressor activity. Both somewhat characterized genes and previously uncharacterized genes are found among them. Because the decrease or loss of gene expression, even if it is combined with the decreased copy number, is not sufficient to demonstrate the functionality in tumor suppression, we will need to proceed to examine the activity by DNA transfection of the expression constructs of those candidate genes.

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APPENDICES

1. Paper
2. Manuscript

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Research Article

Scanning copy number and gene expression on the 18q21-qter chromosomal region by the systematic multiplex PCR and reverse transcription-PCR methods

We examined differences in copy number and expression of 127 genes located on the 18q21-qter chromosomal region of the breast and prostate cancer cell lines, using the systematic multiplex PCR and reverse transcription-PCR (SM PCR and SM RT-PCR) methods that we developed. Semi-quantitative data were obtained that were comparable in quality, but not in quantity, to data from DNA microarray hybridization analysis. In the chromosomal region where losses are frequent in breast, prostate, and other cancers, we detected a homozygous deletion of the SMAD4 gene in the MDA-MB-468 breast cancer cell line. We also observed partial or entire loss of expression in genes such as CCBE1, CCDC11, CD226, NP_115536.1, NP_689683.2, RNF152, SERPINB8, and TCF4 in certain breast and/or prostate cancer cell lines. An increase in gene expression was rare, but found with the transcription factor ONECUT2 gene in all of the cancer cell lines examined. Real-time qRT-PCR experiments confirmed these SM RT-PCR results. Further analysis of clinical specimens of breast cancer by real-time qRT-PCR demonstrated that the gene expression of CCBE1, TCF4, NP_115536.1, and NP_689683.2 was downregulated in the majority of clinical cases of breast cancer.

Keywords:
Chromosomal scanning, gene expression / Copy number changes / Systematic multiplex PCR / Systematic multiplex RT-PCR DOI 10.1002/elps.200700093

1 Introduction

During cancer progression, normal cells undergo many complex changes, both genetic and epigenetic, at either the nucleotide or (sub-)chromosomal level. Oncogenes and tumor suppressor genes play important roles in promoting and inhibiting carcinogenesis, respectively [1]. Proto-oncogenes are activated by gene amplification, up-regulation of transcription and activating mutations, whereas tumor suppressor genes are inactivated by loss of the genes, transcriptional silencing, and inactivating mutations. Therefore, the examination of copy number and expression may help to identify genes involved in carcinogenesis.

Hoping to eventually identify potential tumor suppressor genes of both breast and prostate cancers, we targeted the q21-qter region of chromosome 18 among the chromosomal regions that have been reported to be frequently lost in cancer. In that region, a few genes (SMAD2, SMAD4 and BCL2) have been linked to carcinogenesis. SMAD4, homolog 4 of the *Drosophila* 'mothers against decapentaplegic' (Mad) gene, is a cancer predisposition gene with tumor suppressor activity. The germline mutations of the gene cause the familial juvenile polyposis, which is an autosomal dominant disease characterized by a predisposition to hamartomatous polyps and gastrointestinal cancer [2, 3]. In addition to the germline changes, homozygous deletion of the SMAD4 gene was prevalent in pancreatic carcinomas, and somatic mutations were identified in some of the carcinomas that lacked deletions [4]. Although SMAD4 inactivation was also found with breast, ovarian, and other cancers, it was distinctly uncommon (less than 10%) in other tumor types [5]. SMAD2 and BCL2 are not cancer predisposition genes; however, somatic changes have been revealed. BCL2 (B-cell leukemia 2) is a proto-oncogene and it was cloned from the junction of t(14;18) translocation characteristic of follicular lymphoma [6]. The BCL2 protein is localized in mitochondria and when overexpressed it interferes with programmed cell death independent of promoting cell division [7]. SMAD2, another homolog of the *Drosophila's* Mad gene, may play a role as a tumor sup-

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Abbreviation: SM, systematic multiplex

pressor gene in a small fraction (less than 10%) of colorectal cancers. Therefore, we cautiously assume that additional cancer genes may be present in the region.

There are not many genes in the 18q21-qter region, approximately 140, so we used the systematic multiplex (SM) PCR and SM reverse transcription (RT)-PCR methods, which we developed for semiquantitative analyses of copy number and gene expression [8–11].

2 Materials and methods

2.1 Genomic DNA and cDNA

The following genomic DNA samples were used for the SM PCR experiments of the genes on the 18q21-qter region: a normal tissue and a primary tumor of breast from an individual with invasive ductal carcinoma and its metastasized tumor to lymph node, a normal and a primary tumor tissue of prostate from an individual with prostate adenocarcinoma, primary cultures of normal breast and prostate epithelial cells, and from six mammary (MCF7, MDA-MB-468, MDA-MB-231, BT-20, T-47D, and Hs-578T) and four prostate (PC3, DU145, LNCaP, and MDA PCa2b) carcinoma cell lines. The primary cultures (HMEC and PrEC) were purchased from Cambrex, and the cancer cell lines were originally obtained from ATCC. High-quality DNA preparations were confirmed by gel electrophoresis. Genomic DNA from MCF7, MDA-MB-468, and BT-20 breast cancer cell lines was also used in the arrayCGH experiments.

For the expression analysis by SM RT-PCR, we used the following RNA samples: a normal and a primary tumor tissue of breast from an individual with invasive ductal carcinoma, a normal and a primary carcinoma tissue of prostate, another normal prostate tissue, and a hyperplastic prostate tissue, five mammary (MCF7, MDA-MB-468, MDA-MB-231, BT-20, and T-47D) and three prostate (PC3, DU145, and LNCaP) cancer cell lines, and from primary cultures of normal mammary and prostate epithelial cells. Scarcity of degradation was confirmed with RNA specimens by gel electrophoresis. Total RNA was used to prepare cDNA by RT using oligo dT as a primer and Advantage RT-for-PCR Kit (BD Biosciences-Clontech). These RNA and cDNA samples were also used in the DNA microarray hybridization and real-time qRT-PCR experiments, respectively. Additionally, cDNA samples prepared from 12 matched pairs of normal and tumor breast tissues were used in the real-time qRT-PCR experiments.

2.2 SM PCR and SM RT-PCR experiments to measure and determine copy number and expression of the genes on the 18q21-qter region

The detailed experimental procedures to establish the SM (RT-)PCR system have been previously described [8–11]. Briefly, the genes were categorized into groups of approximately ten genes, and the concentrations of PCR primers in multiplex reactions were optimized to amplify different sizes of DNA fragments in single exons at similar band intensities using genomic DNA from normal human tissues as a control. Genomic DNA and cDNA from the human cells and tissues were used to examine the copy number and expression of the genes on the chromosomal region of 18q21-qter, respectively. After SM (RT-)PCR, small aliquots of reaction products were analyzed by an 8% polyacrylamide gel electrophoresis, followed by staining with ethidium bromide. The gel pictures were taken and saved in TIFF format, the band intensity was measured using the ImageQuant software (Amersham Biosciences) and normalized by adjusting the average band intensities of individual gels.

2.3 DNA microarray hybridization experiments to measure and determine copy number and expression of the genes on the 18q21-qter region

The copy number was also analyzed by DNA microarray hybridization using the arrayCGH method [12–14]. The changes in copy number were examined of three breast cancer cell lines (MCF7, MDA-MB-468, and BT-20) at NimbleGen (Madison, WI, USA). Genomic DNA from normal females was used as a reference for all the three hybridization experiments. Relative fluorescence intensity, which is indicative of relative copy number, was determined over the entire human genome with 385 000 isothermal long oligonucleotide probes tiled through genic and intergenic regions at a median probe spacing of 6000 base pairs.

The expression analysis was also performed by DNA microarray hybridization. Total RNA was used from a normal breast tissue, a normal prostate tissue, primary cultures of normal mammary and prostate epithelial cells, five mammary (MCF7, MDA-MB-468, MDA-MB-231, BT-20, and T-47D) and three prostate (PC3, DU145, and LNCaP) cancer cell lines. The same preparations of RNA that were used in SM RT-PCR were used in the microarray hybridization. Illumina's Sentrix Human-6 Expression BeadChips, which represented probes from the entire 23 000 RefSeq collection and an additional 23 000 other expressed sequences, were hybridized with the biotinylated cRNA that was prepared following the manufacturer's protocol. After hybridization and washing, the BeadChips were treated with streptavidin-Cy3, washed, dried, and scanned for fluorescence intensity, using a BeadStation 500 that was equipped at the DNA Microarray Facility at Burnham Institute for Medical Research. Raw data were generated and then normalized using the Beadscan 3.0 software. The unique 30X average redundancy feature of the BeadChips allows absolute signal detection of a single fluorescence. Other microarrays require the CGH-type hybridization using two different kinds of fluorescence followed by determination of relative signal intensity due a wide variation in the amounts of probes printed on different slides.

Data for the genes and sequences on the chromosomal region of 18q21-qter were extracted from the results of the DNA microarray hybridization experiments and used for comparison with the results from the SM PCR and SM RT-PCR experiments.

2.4 Real-time qRT-PCR experiments to measure and determine expression of the selected genes on the 18q21-qter region

Real-time qRT-PCR was performed for several genes (CCBE1, CCDC11, CD226, NP_115536.1, NP_689683.2, RNF152, SERPINB8, TCF4, and DYM). The ubiquitously expressed DYM gene was used as a control. The subset of the cDNA samples that were used in the SM RT-PCR experiments and additional cDNA samples prepared from the 12 matched pairs of normal and tumor breast tissues were analyzed. The same primer pairs that were used in the SM RT-PCR experiments were also used in the real-time qRT-PCR experiments. Using the Power SYBR Green PCR Master Mix purchased from Applied Biosystems, the reactions were conducted using the Mx3000p system (Stratagene) under the default conditions, except that the annealing temperature was raised to 60°C instead of 55°C. Data were analyzed using the MxPro software installed with the equipment, and the Ct values were obtained for the individual reactions.

3 Results

3.1 SM PCR and SM RT-PCR analyses of the genes on the chromosomal region of 18q21-qter

Using control genomic DNA template, the optimal primer concentrations were determined to unify band intensity as previously described [8–11]. Out of 140 genes on the chromosomal region of 18q21-qter, 13 genes failed to amplify specifically and were excluded from the system. Together with 4 genes in the 18q12.3 region that neighbors the 18q21.1 region and 2 genes in 18q12.2, the SM RT-PCR system consisted of 133 genes in 12 sets (Sets A–L). The list of the genes is shown in Table 1. The nucleotide sequences and concentrations of the primers used in this study are also shown.

We examined the copy number of those genes in breast and prostate cells and tissues by the SM PCR method. Results are shown in the left column of Fig. 1. We found dozens of genes with decreases in band intensity in cancer cell lines, most evidently in MCF7 cells. Complete disappearance of band was observed with the SMAD4 gene (Set B) in the MDA-MB-468 cells, and a drastic decrease in band intensity was observed with ELAC1 (Set C) and PLEKHE1 (Set H) in MDA-MB-468 and RAX (Set F) in BT-20. We also observed an increase, which is suggestive of gene amplification, in the SLC14A1 gene (Set E) in MCF7 among others.

Band intensity was densitometrically measured for the SM PCR bands and the values were input into the table of genes aligned based on their chromosomal locations. The partial results are shown of a normal breast, primary culture of normal mammary epithelial cells, and three mammary carcinoma cell lines in gray scale (black and white for the lowest and highest band intensity, respectively) in the left column of Fig. 2.

We next examined the expression of those 133 genes in breast and prostate cells and tissues. Results are shown in the right column of Fig. 1. The PCR conditions were elaborated so that small amounts of genomic DNA would produce bands. The absence of at least one band indicated the least amount of contaminating genomic DNA in the cDNA specimens. We found that approximately 40% of the genes were ubiquitously expressed in a large amount. Similar band intensities of the ubiquitously expressed genes in the specimens suggested comparable quality and quantity of the cDNA preparations. We also observed that 17 were not transcribed in either normal or cancerous breast/prostate cells/tissues. The other genes were expressed in some, but not all, of the cDNA samples examined. Among them, several genes showed interesting expression profiles. For instance, the expression of CCDC11 (Set C) was completely repressed in three out of five breast and three out of three prostate cancer cell lines that were examined, whereas it was expressed in normal cells and tissues. On the other hand, the expression of the RNF152 gene (Set A) was repressed in prostate, but not in breast, cancer cell lines. Additional genes that exhibited down-regulation of gene expression include TCF4 (Set E), NP_689683.2 (Set F), SERPINB8 (Set G), CCBE1 (Set I), CD226 (Set K), and NP_115536.1 (Set K) genes in a majority of the cancer cell lines and PSTPIP2 (Set A) and KIAA0427 (Set H) in a minority of the cancer cell lines that were examined.

For quantification, band intensity was measured for the SM RT-PCR bands. Together with genomic DNA as a control, the results of a normal breast, a normal prostate, primary culture of normal mammary and prostate epithelial cells, and five mammary and three prostate cancer cell lines are schematically shown in a gray scale from black (weakest) to white (strongest) in the middle column of Fig. 2.

3.2 Comparison of the SM RT-PCR and SM PCR results with the results from DNA microarray hybridization

We compared the results from the SM RT-PCR experiments with the results from the DNA microarray hybridization experiments. We normalized data from the DNA microarray hybridization experiments using the Beadscan 3.0 software. The average fluorescence signal intensities of beads for individual genes on the 18q21-qter region were extracted and gray-scaled, and are shown, side-by-side with the data from the SM RT-PCR experiments, in the right column of Fig. 2.

Table 1. Primers used in the study

Gene Name	Fragment size (bp)	Primer 1 sequence	Primer 2 sequence	Primer conc. (nM)
Set A				
ZNF532	175	TGAAGGGCCTCCAAACTTGGGTAT	AGGACTGGCCACTTTCTTGGTTTC	41
CDH7	160	CTGAGAAACCTCAACGTCATCCGA	CACCAGGATCAACATCGGCTTCTT	205
TNFRSF11A	146	ATGCCAGGATGCTCTCATTGGTCA	TGTGGATTTGCTTCCAGGCTCAGT	41
MBD1	133	TCCAACGAAGCAGGAAGCAGGT	CAACAGGGCTTCTGTGGAAGCTG	102
MBD2	121	CCAGGTAGCAATGATGAGACCCTTT	TGTTAAGCCAAACAGCAGGGTTCT	68
DCC	110	ACTACCCAACAACCACCTATGCTG	AGTGGGTGAGTTGGTCGAACACAAG	102
RNF152	100	TGTCATCGCCATTCCACACACTTC	ACGCTCCTTGGAGATGGGCA	136
CXXC1	91	TGTTTGAGCAGGAGCGCAATGT	GGATCGTGCTGGATCGTCTGGT	170
ONECUT2	83	GAACAAACGCCCGTCAAAGGAGAT	TGAAGAAGTTGCTGACGGTTGTGAG	102
MRO	76	CTGGTGTATGGACTGTATGACCCTGTGA	CCAGAACGACGGTCAGAGTCTTCA	102
PSTPIP2	70	TGAGGCTCAAGAATGTGAACGAATAAACT	TGACAGCTGATTCACATGTAACCACAATGC	102
Set B				
CAD20_HUMAN	175	TGAACAGCACTGTCCACAGCTA	AAGTCGAAGCTCTGTTCCGAGTCC	273
CDH19	160	AGGAGCCTATACAGGCAGTCTTTG	ATCCAGCTAATGACCCTGTTCCCT	68
SMAD7	146	CATCTTCATCAAGTCCGCCACACT	GCTGCATAAACTCGTGGTCATTGG	85
RKHD2	133	CAAGACGAAAGCACGACTGTGTGA	ACTGGACATGATGGCGTTCTCT	55
BCL2	121	AGCATGCGGCCTCTGTTTGATTTC	AGGCATGTTGACTTCACTTGTGGC	68
PMAIP1	110	GAATCTGATATCCAAACTCTTCTGCTCAGG	TCAAATTGATGAAACGTGCACCTCCT	102
SMAD4	100	GCTGCTGCTGGAATTGGTGTTGAT	TGATGCTCTGTCTTGGGTAATCCG	102
MAPK4	91	GACCACGACAACATCGTCAAAGTG	TGTACGCCACGCTGAACTTGAACA	102
SMAD2	83	AGCTTCACCAATCAAGTCCCATGA	AACAGTCCATAGGGACCACACACA	55
RAB27B	76	GCGAATGGAACAGTGTGTGGAGAAGA	CCCATCCAAGTTTCCAGAATTTCCACC	41
PIK3C3	70	CAGAGTCTGATTGATGAGAGTGTCCATGC	GGGCAAACTTGTGAATCTGTTCCACC	41
Set C				
ME2	133	GGAGAGAGAATTCTGGGTCTTGGAGA	TTCCCACATCAATACACACTGGCAGG	100
ELAC1	121	ATGGACAAAGCAAAGGAGCATGGC	AGGCAACTGGTTTGTACCTCTGAC	100
NETO1	110	AGGAGCTACAGCTGACTTTGCAGATG	ACAGCTGTGATCCACAGTGATGGT	100
CCDC11	100	AGGAGCGGAAAGCACAGATTGCAT	GCTAATCGGTCTTCCTCCCAGAGTTT	125
CBLN2	83	GCTCATGGAAAGGGAAGACAAAGTGC	CCGAGAATGTGGAGTATTTCCAGCC	125
C18orf24	76	AGAAGCCTCCCAAAGAGCAAAGA	GAAGGAACACCATTGAACTCATCACAAGT	250
Set D				
SERPINB2	151	TTCAACAAGGGACGGGCCAATTTC	GTCCAGTTCTCCCTGTCATAACACCT	83
ZNF407	138	CTGTACTCCCACACCGTGCT	GGAGCCCTCCTGGGTGTAGATGA	125
GALR1	126	ACAGACAGTTCTGGTGGTGGTTGT	GCGGTGATTCTGAAGAGGAAGGAA	67
WDR7	115	CACCTCAGCTGCGCTGCATTAAA	ACGTTGCGGTTGGAAGTCCAGAT	166
GRP	105	GAAGCTGCAAGGAATTTGCTGGGT	TGAATCCCACGAAGGCTGCTGATT	83
ALPK2	96	AGTTTAAAGCACTACACCAGTGTAACAAGT	CAATGCTCGGCTGCTTCTGTTTCT	166
LIPG	88	ACTTGGGAGACCTCTTGAAGATCCAG	AGGTAGCTGCGAAACTCCTTCCA	83
SLC39A6	75	GTTCTACTAAAGGCTGGCATGACCGT	AAGATACGCCAGCATGGCTGACAA	166
GALNT1	66	TCAGTGCCTGGATAAAGCCACAGAAG	CTTCCATTGCAGTCTCTAATGCTGGG	125
Set E				
XP_372695.2	192	GCATACCCAAGGACAAGGCCATTA	TGCGGGCTACACAAGATTGATTCC	58
POLI_HUMAN	176	TCTCCTTGTGAACCGGGAACATCA	AAAGCAGACACAGCAGGGTTTGAA	115
Q96N33_HUMAN	161	TGGCCATGACCCACATGAGGATTT	TGAAACAGAAGATGAACTTGATGACCAGGG	58
Q8TCD1_HUMAN	147	GGTGCAGACATGAATGGATTACCAACA	AGGTCAGAGACAATTACAAGGAAGATGC	87
Q7Z5E4_HUMAN	134	ATGCTCTGGCGTCTACTGCATTTC	GACACCCAATTCCTTCACTTGCCA	87
SLC14A1	122	CTTCTGTTTGGCCACGCTATTGTTCC	TCTTGGCTTGCAGGTAGAAGATGC	115
TCF4	111	ATGAGGACCTGACACCAGAGCAGAA	GCTCTTTGAAAGCCTCGTTGATGTC	144
PIGN	101	TTTGGTGTTCCTCAATGGCCTGG	TGGTGCTTCAGCAACCTCACAT	115
FVT1	92	GGTGGGCAGGATCGTGTTTGTGT	CCCTTATGGCAAACTTGGATGCAG	115
NP_001008240.1	77	GTTAGCACCAGGCAAACACAGTCA	TCAACAACTTCTGCTGGTGAAGCC	115

Table 1. Continued

Gene Name	Fragment size (bp)	Primer 1 sequence	Primer 2 sequence	Primer conc. (nM)
SERPINB7	71	AGATTCTTGAGCTCAGATACAATGGTGGC	TTCAGAGAGGTCATTCTCAGGCAGC	87
KATNAL2	66	ACTGCCGACTTTCTGGATGTGCTA	GTATCTCTGAGCCAGATTCTTTGCGG	87
SEC11L3	62	GAGGCTTGTACAAAGAAGGCCAGA	TCTTGCTCTTCCCACCACGTCCTT	115
Set F				
XP_113971.3	193	AAATCCACATGCGGAAGCACACAG	CAGCTCTGGCGCTTGATGTGGC	188
ENSG00000188451	177	CACACCCTGACACAGCTTATTTCTGC	ATCTCCAGTAACTTTGCCACCCTTC	63
TCEB3C	162	TCTGGCCACTAAGACGGAGCCGAAA	GCCGCTAAGTCTCTGGCAAAGT	38
NP_005594.1	135	CAGAAGCATCGCAAGCGGTTGAA	GGAGGAGATGAGGTCCGCGTAG	125
VPS4B	123	TAGGGACCACTCAGAACAGTCTCA	TCCTAACAGGCTGCATAAGGGCAT	63
STARD6	112	AAACCCAGCATATTCCAAACTAGTGATGT	GAGGATGAAGTTTACTAAGTTGGAAGGCA	94
KIAA1468	93	TTGCTGCAAGCTTAGTGAGTGAAGA	ATACATTGGCCAACATGGCACCTG	125
NP_998767.1	85	TCCCTCAGCCACCAACATCCATTT	GAGAACCAGGACTGGCTGTGCC	94
TXNL1	78	GTTCAGTCGAATCAAGGTGAAGAGGA	TGTTGCCTGGACTGGAGTACCAAT	125
RAX	72	GACAAGTTCCCGCTGGACGAGG	CTCCTTGGCTTTCAGACGCAGC	188
SERPINB10	67	ATCCAGAATCTCCTGCCTGATGAC	ATAGGGCGTTCACCAGAATCATCC	94
NP_689683.2	63	AGTGGCTCGCCATGAGCAAGAAAT	CTCCCAGTTGTGTCTCAATGTCCACT	94
Set G				
ZCCHC2	194	GAACACGAACGCTAATGGGACAGT	TGCCATTGCAAATGGATGGCAGAG	87
XP_371118.1	178	GATCAACGAGGAAAGCGACTACCA	CAAGGCTTCATTCTCTCGCTGGAA	87
C18orf12	163	TTCCTCCAACTGCATCGCTCAATC	CTCCCACTTTCAGCATTCTGGCTT	87
LOXHD1	149	TCTTTAACTGTGACTGCCTCATCCC	CTCATAGCCTGTTGTCACGATGACTT	87
NP_066015.1	136	GTGGAGGAAGAGGCAAAGCTGTTT	GGTTCTGCCTGCTCTGAACCAAGA	87
CPLX4	124	TGGCTGGAGATGATGTGGATTTACC	TCCAAGTCCATGTTCTGGAGATTCTG	87
HDHD2	113	GCACCTCTGATAGCAATCCACAAAGC	GTGGCTTTGGTATCTGTGGCATACTC	46
C18orf54	103	GCCAAGAGAAATCTAGAGCAGTGTACTGAA	CCCATGATCTGTCTGCTTCAAGTTTATCT	87
SIA8C_HUMAN	94	ATTTACCACCAAGTGGCAGGAGTC	CAGAGTCAGCTTGGTGAGCCCTT	173
ACAA2	86	CCCATGGCAATGACTGCAGAGAATC	TGCTGTGACTGCAGGGCATATT	58
SERPINB12	73	GGCACAGATCCTGGAAATGAGGTA	TTTAGAGTGAGATGGCAGCAGCAC	58
SERPINB8	68	CAGAAGTTCTATCAGGCAGAGCTGGA	TGCTTCCTGCACTCTTCAGTGTCT	115
NEDD4L	64	TCGCCTTGACTTACCTCCATATGAAACC	CACGGCCATGAGAAGTTTCTCTCGTA	87
Set H				
PLEKHE1	195	AAGGAGAAGGAGAAACAGCAGCACCT	CACCTCTATCACATTGTGGCTCCT	115
SIA8E_HUMAN	179	TTCCACAAGCTGGAGAAGTGGC	TACTGCGGATGGAAGTAGTAGACAGC	144
MALT1	164	GAGGACAAGCAGGAAGTGAATGTTGG	TGCAATGAGTGATAATGCCCTGCTCC	46
C18orf26	150	TGGCTTGTCTCTTAGCCTGTGTGA	AGGGTGTTCCAGGTTTGACAGT	46
KIAA0427	137	ACAGCTGCCTGAGATGATGACAGA	GCGTCAGAGGGTTCCAGCTGTTAG	58
MC4R	125	GACTCTGGGTGTCATCAGCTTGTT	TCACCAGCATATCAGCCACAGCCAA	46
MYO5B	114	TTCACCAGAGTGGAGCAGTTCAGA	AGGTACACAGGGAGCAGATAGCCT	87
ATP5A1	104	AAGTGGCTGTTATCTATGCGGGTG	CTGGCTGACGACATGAGACAAGAA	58
LMAN1	95	AACCGTCAGACTGGTCAGTGGAAT	GCAGGTGCTCTTTGATGTCAATGAAGTG	87
SERPINB13	87	GATGGCTCTATTAGTAGCTCTACCAAGCTG	TTCTTTCTTAAACTCCCTGTCCCATTGCC	115
FECH	80	ATCCAGTCAAACGAGCTGTGTTCC	TTAGTCTCCCTGCAGACAGGATTGAC	115
IER3IP1	69	GTGGATTTGGAGAAGAGCCGGGAAT	TCACGGTTCTTACAGATCGAATAAGGTTCA	144
C18orf20	65	AGGTTACAACCAGAGACCTGAAGGA	TCCTCTTCTCTTTGACAACCATGTGGC	173
Set I				
ENSG00000182288	232	TGCTGCTCAAGCTCCATTCACGA	GCCAGAGACAGGCATGAGAGATCAAA	50
Q8N7F0_HUMAN	214	TTTGGAAAGGAACATAAATGACCTGACAGA	TTTAGGTCTTTGAGAAATTGCCACAGTGTT	94
Q9H380_HUMAN	197	CCTGCATGGATTTGCATGTTTCCC	AGGTTAGAAGAATAAGAAAGGGAGTCTGGA	125
CCBE1	181	CCTGGTTCTTTCGACTTCCTGCTA	TTCTTGGATGGTCATCTCCAGAGCC	63
NP_079490.1	152	GGAAATGAACTGGCTGGATGAAGATCTGA	TCATCTTCTTGCAAAGCCATTGGTATGT	188
NARS	139	AAGGTGGTGCCACACTCTTCAA	TGCTCTGCCCGGTATGACTGAGCAAT	94
PIAS2	127	ACCCTTAACAGCAAGCAGTACGTC	GTCAGGAATGTTACTTCCACTGCTGG	94

Table 1. Continued

Gene Name	Fragment size (bp)	Primer 1 sequence	Primer 2 sequence	Primer conc. (nM)
SERPINB4	116	TGGAAGAGAGCTATGACCTCAAGGA	TTTAGATACTGAGAGACCGTGGCTCC	94
DYM	106	CAAATATGTGGAAGAGGAGCAGCCC	CTGGATGTCCTGTGGATTCCAGT	94
CCDC5	97	TCAAGACCTTCTCATGGAGAGTGTGAA	GGCCACCGCACTGTCAACCAAA	125
ENSG00000141690	89	TTGGACTTCCATCATCCTCATCAACTACT	AACTGTTCCAGAGATTCAGGGTGG	94
C18orf24	82	ACCCGTAAAGAAGCCTCCCAAAGA	AGGAACACCATTGAACTCATCACAAGT	94
Set J				
NP_055728.1	235	ACTGGTGCCTGTGTATGTGAAGGT	AGCCAGGGTCATATTTCCCGTGTA	46
SALL3	200	ACAACGAGATCTCCGTCATCCAGA	ATCCTCGATAAACCGCGTGAATGG	87
ENSG00000196512	169	AACCCAGAATGCCTCTTCTCCTCT	AGCTTCCTTGCACTGGGCTATAAG	58
SOCS6	155	TGGATCAGTCCGTGAATGGCTTGT	TGTGCCAGTGAGTCCACTGAAGTT	46
PQLC1	142	CAGTGGAGCAGCTTCTCGGACTACG	CCAGCATGGCTTCGGTCAGCACAG	115
CNDP2	130	GTTGAGCCAGACTTGACCAGGGAA	TTTCATTCTGGGAGTGGGCTCCGT	87
Q8N8S9_HUMAN	119	CTCTTGTTCCCAGGCCCATCCAGC	AATCCGAAGGAGGTTCAGGGACTG	173
NP_997344.1	100	TAATCATTGGCTGCCTCCACTCCA	CTTGACGGCTGTCATCAAACAGGT	87
NP_872376.1	92	AAAGAGGGAGAGAAGGAACCAGGCT	AGGAACCTGGCCCTTCGGAAGTCT	87
NP_079057.1	85	CTGAATCAGATCCGTAAGCTCCAGAGG	GCAAGTGCCTGAGTTCAGTCTCTAAGT	87
ENSG00000182671	79	ATTGCCAGAAAGAACCTGGCTTGC	AGCAAGCCTAATGAAGAGGCTCCA	58
ENSG00000176594	74	AATACTTCCTTGGTCTGTTGGGCCAT	TCAGAGCCAGCTGCTTAAGGAATGTG	58
CYB5	70	AAGCTGGAGGTGACGCTACTGAGAAC	TTGGACATTTCCCTGGCATCTGTAG	87
Set K				
NP_115536.1	185	GCCTTTCTTGGAATTCCTTTGTCTCCTGC	CGATCCATCAGAGTCCAGCAGATGTT	63
ZADH2	170	TTAAGCAGGAGTACCCTGAAGGTGTC	CAATGTTCCTGCTTTCACAGGCGA	94
ZNF236	156	CGGCCGTTCCATTGCACGCTTTGT	CCGCTTCATGTGCAGCTTCATGTT	50
CD226	143	GCCACATTGTTTCGGAACCTGGAA	TCTGCCATGGACCAAGTTGCAGTA	63
TXNL4A	131	ACAAGATTAACTGGGCCATGGAGG	TCAGTAGCGGTACTTGGTGGAGTA	94
ATP9B	120	ATCTCCTTCACCGCACTGATCCTGA	GAGTGAGGACACGTAGCAGCCTAA	125
PARD6G	101	AATGACGAGGTCCTGGAGGTGAAC	GTGACGATGAGGTTGTGGCTGTTG	312
GTSCR1	93	ACTCATCTACTGCAAGCTTGGCCC	ACTGACCATAGAGATGGTAGTGATGTCT	63
CTDP1	86	CAGATGTTTGGTGAAGAGCTGCCT	TCAGACATACTGGGCTGTCGCTTT	94
Q9NY04_HUMAN	80	CCAAACTGCCATTCCAGTCACTCA	CCTAGTAGAACAAAGAAAGCCCTGGAA	125
CNDP1	75	ATGATCCGGGATGGATCCACCATT	AATTAGCACCACGCTCTTGTGGAC	94
FBXO15	71	ACCCTCTGACAGCTCTAGCTTCTT	GCACTCTTCCTTCCGCATCAACGTA	94
Set L				
SDCCAG33	219	GCAACGATTGTGCCTCTCAGTTCA	TCCGGTTGCAGAGCTTACATTGGA	83
TXNDC10	171	GTGCTATGGAATCTACACAGCCGA	AATACATCCTTGGGCTCCTGCACT	67
NP_079081.1	132	CAGCTCCCTCAAGAGTTACCTGTCA	TCTGTTCTGCCACCTCCTCTCTCT	125
Q96MY0_HUMAN	121	AAAGGTGCCATGCCAGAGAGATGA	AGGACAGAAGCAGTTTGCTGATGC	83
NP_997343.1	111	CAATCCTGGCGGTTACCTCAGCGG	CAGCGCGTCTGGAGTAGTTTCTTT	125
NP_054896.1	102	CGTACAGTATACGGAGAAGCTGCACA	CTCAAACTGGGCTCAGTCTTCAAGCA	167
XP_058931.6	87	TGGTGGCTATTGATGTGGACATGG	TGCAGTCTTCATCTCCTGTGCAGT	83
RTTN	81	CCCAAACTCAGAAGCAAACCCTCT	CAGGAAGAATTAAGGAGCTGCACGAG	167
MBP	72	AAGGCCAGAGACCAGGATTTGGCTA	CCTTGAATCCCTTGTGAGCCGATT	208

We also compared the results from the SM PCR experiments with the results from DNA microarray hybridization experiments. We had not established the arrayCGH system in our laboratory, so we outsourced the arrayCGH hybridization experiments to NimbleGen, one of the pioneering providers of commercial services of the technique. We submitted the same genomic DNA from MCF7, MDA-MB-468, and BT-20 breast cancer cell lines that was used in our SM PCR experiments. Data for the probes corresponding to the sequences on chromosome 18 were extracted, and $\log_2$ values of relative fluorescence intensity to normal female genomic DNA were plotted on the *Y*-axis along the chromosomal location on the *X*-axis (pter to qter from left to right). Results are shown in the upper panel (a) of Fig. 3. Similarly, $\log_2$ values were calculated of relative band intensity of those cell lines to normal female breast from the SM PCR data in

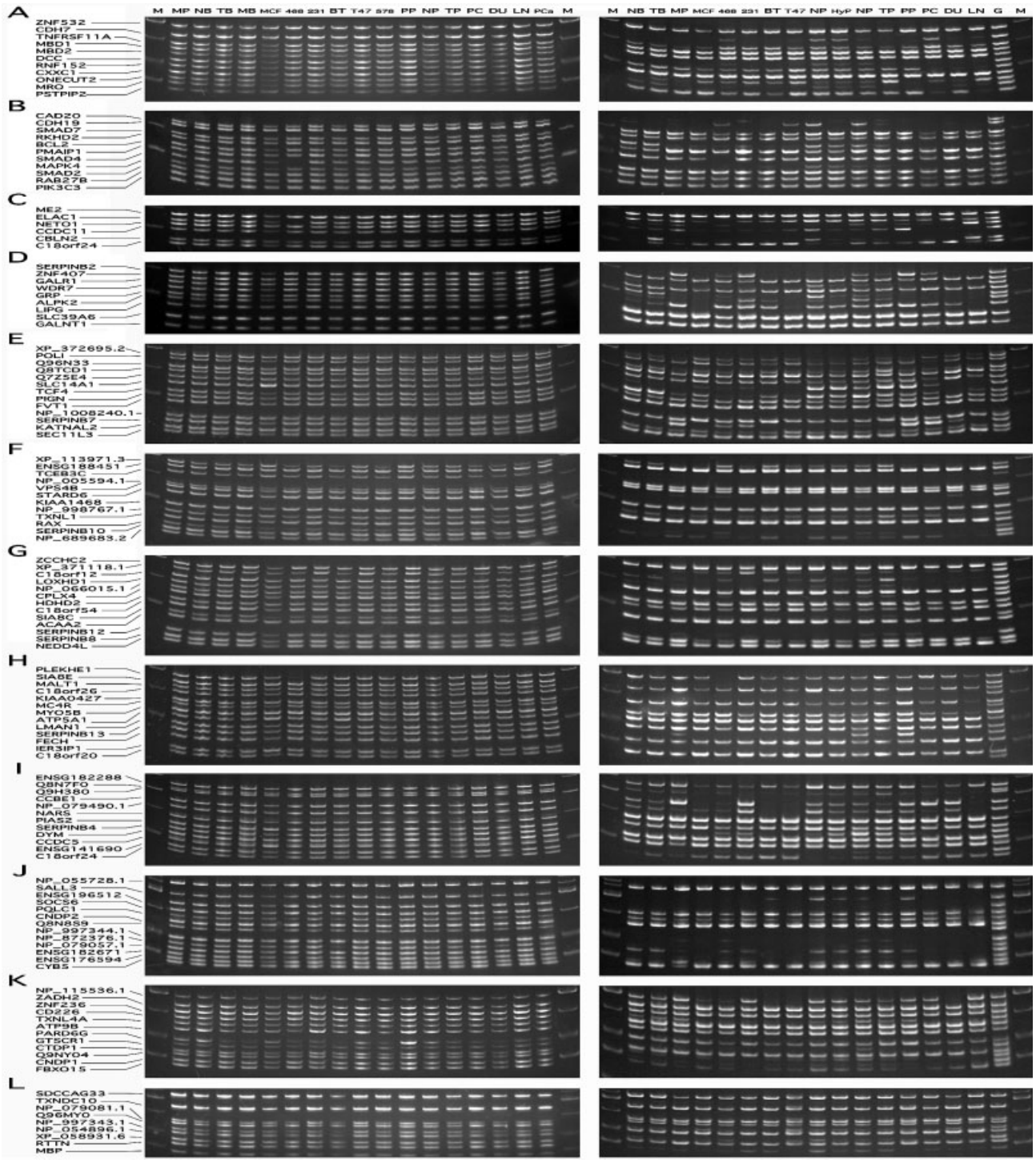

Figure 1. SM PCR and SM RT-PCR results of breast and prostate cells and tissues. The left and right panels show the results of the SM PCR and SM RT-PCR experiments, respectively. There are a total of 12 sets (A–L). SM PCR and SM RT-PCR were performed to examine copy number and expression changes in breast and prostate cancer cells and tissues. The sources of genomic DNA and cDNA are abbreviated: a normal sample (NB), primary tumor (TB), and metastasized tumor (MB) of breast tissue from an individual; a normal sample (NP), and primary tumor tissues (TP) of prostate from an individual; a normal prostate tissue (NP) from a third individual; a hyperplastic prostate tissue (HyP) from a fourth individual; primary cultures of normal mammary (MP) and prostate (PP) epithelial cells; and MCF7 (MCF), MDA-MB-468 (468), MDA-MB-231 (231), BT-20 (BT), T-47D (T47), Hs-578T (578), PC3 (PC), DU145 (DU), LNCaP (LN), and MDA PCa2b (PCa) cancer cell lines. The locations of the DNA fragments amplified from the individual genes are also shown at the left side of the gel pictures. The symbol M denotes DNA fragment size markers, and the symbol G shows the results of genomic DNA control in the SM RT-PCR experiments.

Figure 2. Intensities of the bands amplified by SM PCR and SM RT-PCR and the intensities of fluorescence detected after DNA microarray hybridization of the genes on chromosomal region of 18q21-qter. Data that were obtained by the SM RT-PCR and SM PCR experiments that are shown in Fig. 1 were used to prepare this table by the densitometry measurement of band intensity. The average band intensities of individual gels were adjusted to normalize the values. The partial results of the SM PCR and SM RT-PCR experiments are shown in the left and center columns, respectively. The values of the band intensities of the PCR-amplified fragments were aligned by their chromosomal locations, and are shown in gray scale, with white as the strongest and black as the weakest. Data on fluorescence signal intensity were extracted for the genes in 18q21-qter from the DNA microarray hybridization results, normalized, and aligned (shown in the right column). The negative values obtained by microarray hybridization were recorded as zero in the table. The gene names, cytobands, the start and end of the gene locations, and the primer sets, are also shown.

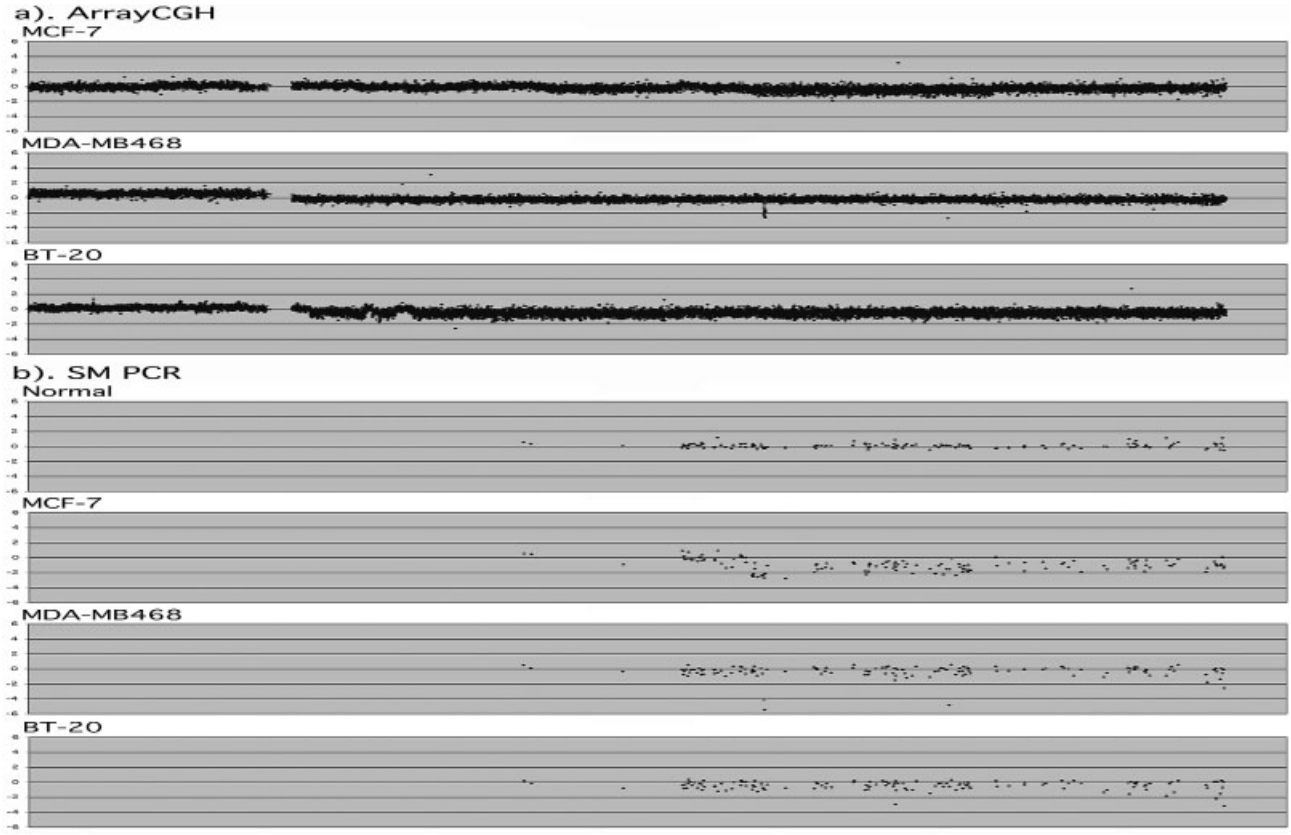

Figure 3. Comparison of the SM PCR results and the arrayCGH results. Data for the genes on chromosome 18 were extracted and normalized from the arrayCGH experiments of three breast cancer cell lines, MCF7, MDA-MB-468, and BT-20. The $\log_2$ values were calculated and plotted on the *Y*-axis with the chromosomal location on the *X*-axis in (a). The rightmost end of the *X*-axis corresponds to 80 000 000 base pairs from the pter. Since there are only 76 117 153 base pairs in the chromosome, there is a gap between the qter of the chromosome and the ends of the graphs. The relative band intensity was calculated by dividing the band intensity values shown in Fig. 2 of the genes for normal mammary primary cells, MCF7, MDA-MB-468, and BT-20 breast cancer cell lines by the corresponding values for a normal breast tissue. The $\log_2$ values were then used to plot on the graph in (b).

Fig. 2 and were plotted. Results are shown in the lower panel (b) of Fig. 3. Except for the number of data points, the graphs were similar between the SM (RT-)PCR and arrayCGH results. Both showed that the copy number was constant over the 18q21-qter chromosomal region in MDA-MB-468 and BT-20 cells, whereas there were at least two changes in copy number in the region in MCF7 cells: one around 46 Mb and the other around 60 Mb from pter. Additionally, homozygous deletion of SMAD4 gene was recognized by those two methods.

3.3 Comparison of the real-time qRT-PCR results with the results from the SM RT-PCR and DNA microarray hybridization

To confirm our findings by the moderately high-throughput SM RT-PCR and high-throughput DNA microarray hybridization expression analyses, we next performed real-time qRT-PCR for the promising candidates of cancer genes: CCBE1, CCDC11, CD226, NP_115536.1, NP_689683.2, ONECUT2, RNF152, SERPINB8, and TCF4. As a control, we examined the expression of the DYM gene. This gene encodes Dymeclin (Dyggve-Melchior-Clausen syndrome protein) [15] and both the SM RT-PCR and the DNA microarray hybridization experiments showed ubiquitous expression in large quantity for all of the cells and tissues we examined. The $\log_2$ values of band/fluorescence intensity were calculated for SM RT-PCR and DNA microarray hybridization, using the data in Fig. 2 and plotted against the Ct values obtained by real-time qRT-PCR. The results were compared and are shown in Fig. 4.

Figure 4 clearly demonstrates a better correlation between the results of SM RT-PCR and real-time qRT-PCR than between the results of DNA microarray hybridization and real-time qRT-PCR. This is reasonable because both SM RT-PCR and real-time qRT-PCR are PCR-based techniques and the same pairs of primers that were proven useful in the SM RT-PCR were used in the real-time qRT-PCR experiments. Based on these results, we concluded that the differences in gene expression that we observed by SM RT-PCR were real.

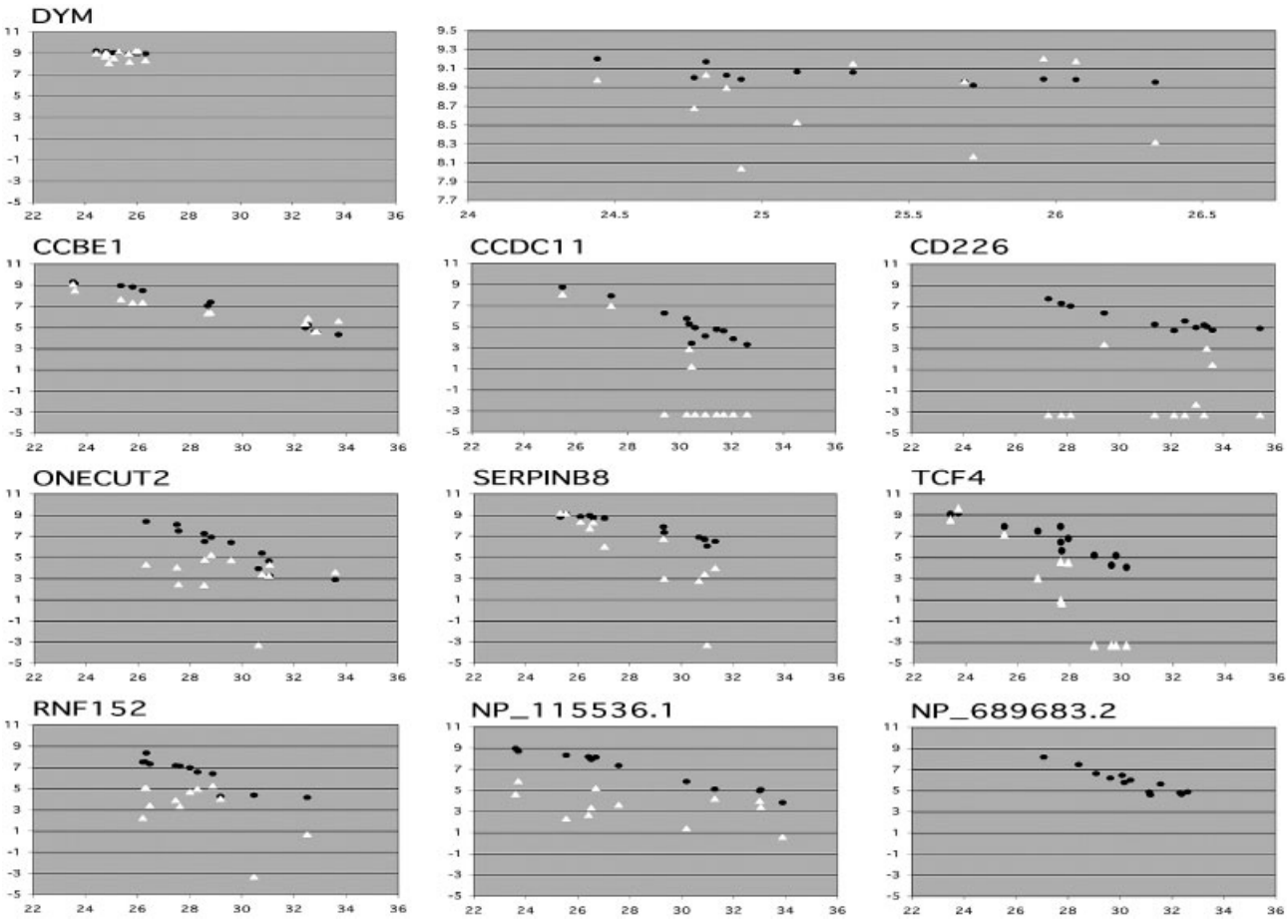

Figure 4. Correlation between the band intensity obtained from the SM RT-PCR or fluorescence intensity obtained from DNA microarray hybridization and the Ct values obtained from the real-time qRT-PCR experiments. The $\log_2$ values of the band intensity (closed circle) or fluorescence intensity (open triangle) were plotted along the *Y*-axis against the Ct values on the *X*-axis. The DYM gene was used as a control because this gene was ubiquitously expressed in large quantity in all the cells and tissues that were examined in both the SM RT-PCR and the DNA microarray hybridization experiments. Negative and zero values obtained by microarray hybridization experiments were assigned the value of 0.1 for these graphs. The portion of the DYM results was enlarged and is shown in the right graph on the top row.

3.4 Real-time qRT-PCR of clinical specimens of breast cancer

As the next step, we performed real-time qRT-PCR using cDNA prepared from clinical specimens of breast cancer. Cancer cell lines provide a useful starting point for the discovery and functional analysis of genes involved in cancer. The alterations found in cancer cell lines, however, may not necessarily be present in the original tumors. Those changes may have been acquired during a long cultivation *in vitro*. Therefore, it was necessary to evaluate whether the same differences are also observed in clinical specimens of cancer in addition to cancer cell lines. We did this using cDNA prepared from matched normal and tumor pairs of breast tissues. The expression of the DYM gene was used as a control to normalize the expression levels. The subtractive Ct values were plotted of the matched normal (on *X*-axis) and tumor (on *Y*-axis) pairs of breast tissues and the partial results are shown in Fig. 5. Downregulation of gene expression was observed with the CCBE1, NP_115536.1, NP_689683.2, and TCF4 genes in a majority of clinical cases of breast cancer (11, 9, 9, and 11 out of 12 cases). A reduction of greater than 50% was observed in 8, 5, 7, and 9 cases, respectively.

4 Discussion

Using a model SM (RT-)PCR system that contained genes from autosomes and the X chromosome, we previously demonstrated that less than a twofold difference in copy number could be detected by SM PCR [11]. In the present study we applied SM PCR and SM RT-PCR to examine the changes in copy number and expression of more than a hundred of genes in breast and prostate tumors and cancer

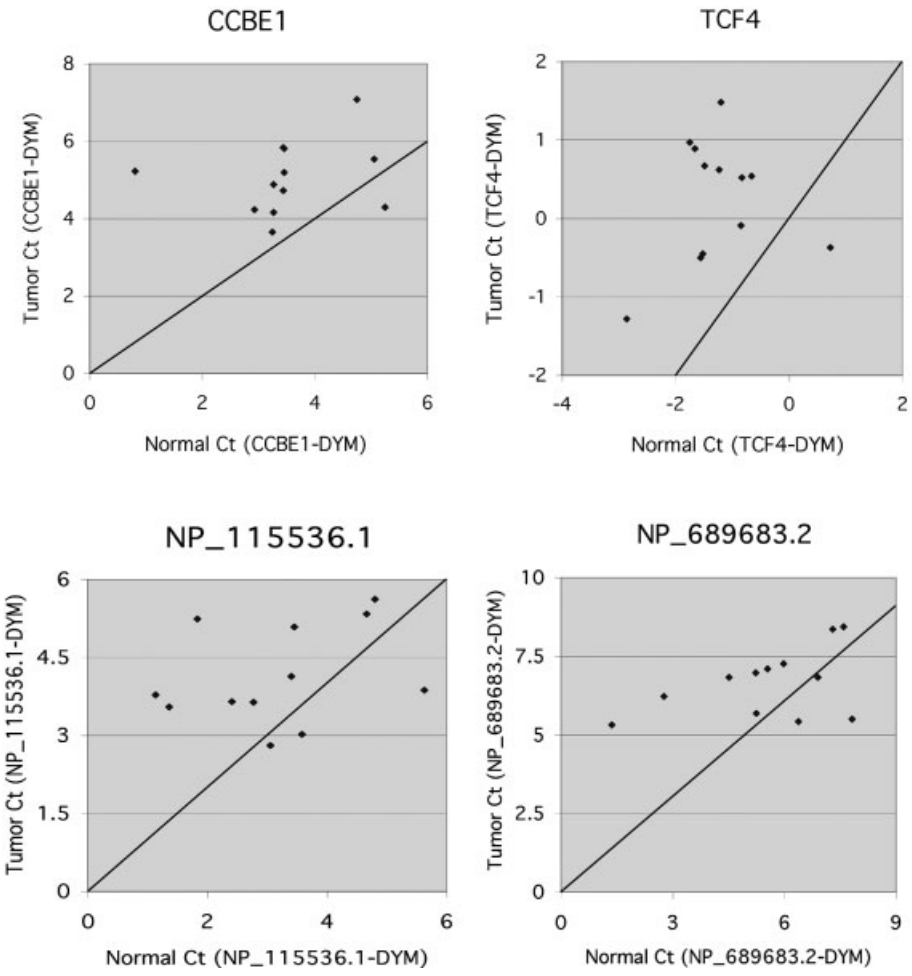

Figure 5. Expression of the selected genes in matched normal and cancer breast tissues. The gene expression was determined for the CCBE1, TCF4, NP_115536.1, and NP_689683.2 genes by real-time qRT-PCR using cDNA prepared from 12 matched normal and cancer breast tissues. The expression of the DYM gene was used to normalize the expression data. The subtractive Ct values (minus Ct DYM) of normal tissues are mapped on the *X*-axis and those of the corresponding tumor tissues from the same individuals are on the *Y*-axis. The line $y = x$ is also shown. The dots above the line indicate downregulation in tumor, whereas dots below indicate upregulation.

cell lines. The total number of genes analyzed by SM (RT-)-PCR was 133 and exceeded the number of genes analyzed in any one of our previous studies. We focused on the genes on chromosomal region of 18q21-qter because loss of this region has been repeatedly observed in breast and prostate cancers, and tumor suppressor genes, whose inactivation could contribute to the development of breast and prostate cancers, have yet to be identified.

We observed a striking increase in band intensity with the SLC14A1 gene in MCF7. SLC14A1 is a member of SLC14 gene family of urea transporters [16]. Although the copy number increase of the SLC14A1 gene is associated with an increased gene expression, this difference is unique to MCF7, and therefore does not seem to be common phenomenon. Both the SM PCR and arrayCGH methods failed to detect the sequences of the SMAD4 and ELAC1 genes in the MDA-MB-468 cell line. This is in line with the expression studies since scarce or no expression is found in the SM RT-PCR and microarray experiments, suggesting homozygous deletion of the genes. Homozygous deletion was previously reported of the SMAD4 gene in pancreatic cancer [4] and of the ELAC1 gene in a lung carcinoma cell line, Ma29 [17]. The results differed between the two methods with the RAX and PLEKHE1 genes. RAX is a paired-type homeobox gene, and the CpG island associated with the RAX gene promoter was found methylated in melanoma [18]. We observed considerably decreased signal only by the SM PCR method. However, the functional significance is not clear because the gene was rarely expressed in normal and cancerous breast and prostate cells and tissues. The PLEKHE1 gene, which is also called PHLPP, encodes a PH domain leucine-rich repeat protein phosphatase that specifically dephosphorylates the hydrophobic motif of Akt protein kinase, promotes apoptosis, and suppresses tumor growth [19]. An additional screening showed that MDA-MB175-VII, another breast cancer cell line, also failed to amplify the SM PCR band from the gene, in addition to MDA-MB-468. However, the following DNA sequencings determined that rather than a homozygous deletion of the PLEKHE1 gene, a 3-nucleotide deletion (GCA at nt 4743–4745) in those cell lines in one of the two primer sequences used in the amplification was responsible for the disappearance. The difference, which causes the

deletion of one of the two glutamines at amino acids 1580–1581, was not found in Ensembl or GenBank SNP databases and its functional significance remains to be elucidated.

The first difference we observed between the SM RT-PCR and DNA microarray hybridization experiments was that the results from the DNA microarray hybridization experiments exhibited a tendency to scatter to the lowest and highest extremes unlike the results from the SM RT-PCR experiments. This is reasonable because a wider linear range of signal detection by the hybridization method allowed the detection of stronger signals without saturation and also because PCR-based SM RT-PCR could detect signals from rare transcripts by amplification. The second difference was that there were eight genes whose expression was not detected by the hybridization method, but was detected by the SM RT-PCR method. For some of the genes it is possible that DNA fragments amplified by SM RT-PCR and the oligonucleotide probes in the BeadChips were derived from alternatively spliced different exons or that the expression was too low to be detected without amplification. However, we suspect that inadequate probes that were not pre-tested may have caused some of the false-negative results. In the SM RT-PCR experiments, we used normal genomic DNA template, which contains all the genes, to establish the system. The useless primers, which failed to amplify the expected sizes of DNA fragments or produced additional bands, were excluded. The problem with the DNA microarray hybridization method is that not all the probes in DNA microarray have been tested for their utility.

In contrast to copy number analysis, we observed more differences in gene expression. Decreased expression in tumors and cancer cell lines was observed with both known protein-coding genes, as well as uncharacterized potential genes. The known genes include coiled-coil domain-containing protein 11 (CCDC11), RING finger protein 152 (RNF152), T cell-specific transcription factor 4 (TCF4) [20, 21], cytoplasmic protease inhibitor 8 (SERPINB8) [22], collagen and calcium binding EGF domains 1 (CCBE1), CD226 antigen precursor [23], and PSTPIP2 that regulates F-actin bundling and enhances filopodia formation and motility in macrophages [24]. The uncharacterized genes include NP_689683.2, NP_115536.1, and KIAA0427. The down-regulation was confirmed for CCDC11, RNF152, TCF4, SERPINB8, CCBE1, PSTPIP2, and KIAA0427 by microarray hybridization, whereas the expression of CD226 and NP_115536.1 was undetectable in certain normal cells/tissues, in addition to several cancer cells, by the microarray hybridization method. The result of hybridization was not available for the NP_689683.2 gene. Consistently increased expression in cancer was only observed with the ONECUT2 (OC2) gene, which encodes a transcription factor characterized by the presence of a single "cut" domain and an atypical homeodomain [25], in the 18q21-qter region by SM RT-PCR. The increase was not so obvious by microarray hybridization, possibly because of its low level of transcription.

Real-time qRT-PCR confirmed that all the above-mentioned differences in gene expression of the cancer cell lines were real. However, only a subset of the genes survived candidacy after real-time qRT-PCR of clinical specimens of breast cancer. Downregulation in expression was observed for the CCBE1, TCF4, NP_115536.1, and NP_689683.2 genes in 11, 11, 9, and 9 out of 12 breast tumors, respectively. The results also showed that the CCBE1 and TCF4 gene were the most promising candidates among those examined, because decreased expression in tumor was observed at the highest frequency in breast cancer cases. As opposed to TCF4 whose link to breast cancer has recently been suggested [21], little is known about the CCBE1 gene and protein, except that the amino acid sequence of the CCBE1 protein predicts the presence of a signal peptide and collagen and calcium binding EGF domains. Because these domains are found in some of the extracellular matrix proteins, the loss of CCBE1 protein expression may result in changes in cellular characteristics, such as adhesion and motility. Further study is underway to pursue this possibility. The difference in gene expression was not so obvious with SERPINB8 (7/12 downregulated) and ONECUT2 (7/12 upregulated). Surprisingly, more tumors were found to exhibit increased expression than decreased expression with the CCDC11 (10/12 up), CD226 (9/12 up), and RNF152 (8/12 up) genes, in contrast to the decreased expression observed with the cancer cell lines. Heterogeneity in cellular constituency of tissues and contamination of normal cells and infiltrating lymphocytes in tumor tissues may have contributed to the results. The different environment that surrounds the cells *in vitro* and *in vivo* may have affected the gene expression. However, the discrepancy may also be explained by the acquisition of downregulation of the genes after the cancer cells were brought into the *in vitro* culture.

For the three previously identified cancer genes, we have mentioned SMAD4 above. Not much change was observed for SMAD2 in copy number and gene expression. For BCL2, the expression was found lower in both normal and cancerous cells in comparison with normal breast and prostate tissues. Whether cells other than epithelial cells are responsible for higher expression in those tissues or loss of 3-D architecture shut down transcription is a question that needs to be answered. In either case, it is unlikely that BCL2 plays an oncogenic role in breast or prostate cancer, as opposed to follicular lymphoma in which it does.

We started SM PCR and SM RT-PCR methods when the DNA microarray hybridization technique was still in its infancy. Only dozens of laboratories were successful in producing meaningful results. During the past several years, significant progress has been made. Commercial DNA microarrays with high quality have become available and the companies that perform custom hybridization have appeared. Concerns were raised about the reliability of DNA microarray results because the results varied con-

siderably among different platforms and different laboratories [26]. To improve the cross-platform concordance and to minimize the variation among laboratories, the MicroArray Quality Control (MAQC) project was launched that recommended the use of standard RNA samples for comparison. Thanks to those efforts, DNA microarray techniques have been maturing. The results of the copy number analysis done at NimbleGen were satisfactory, although the turn-around time of 6 weeks was longer than we expected. The cost was reasonable. However, it was too expensive for us to order hybridization experiments with all 17 specimens analyzed by SM PCR, and therefore, we outsourced only the 3 hybridization experiments with the cell lines that were shown to exhibit significant changes in copy number by SM PCR. The number of detection points differed drastically between the two experiments. Whereas a little more than 5000 probes were examined over 18q21-qter with 6000 base pairs interval by the arrayCGH method using the NimbleGen microarrays, only 134 detection points were examined by the SM PCR method. In spite of this huge difference in number, both results exhibited impressively similar patterns of copy number changes. DNA microarray hybridization experiments for genome-wide gene expression were performed at the DNA Microarray Facility at the institute. The number of the probes of the Sentrix Human-6 Expression BeadChips was 47 293 and smaller than the number of the probes in the NimbleGen microarrays (385 000). Although each probe was represented by an average of 30 beads in the BeadChips, the confidence level may have been higher with the NimbleGen microarrays because the probes, which failed to hybridize with reference DNA, were excluded from consideration in the copy number analysis by arrayCGH. Compared with the hybridization using commercial DNA microarrays, establishment of the SM (RT-)PCR system is laborious and time-consuming. Because the results are obtained in multiple sets, there is a variation in the results among different sets. However, the situation may not be much different from DNA microarrays where the amounts of DNA printed may vary among different probes.

In the present study, we demonstrated the utility of the SM PCR and SM RT-PCR. We also verified that the results obtained by our methods were comparable in quality to the results obtained by DNA microarray hybridization method, although they were not identical. The ability to identify the amplified fragments by their size may counteract the deficiency of nonlinear amplification of signal by PCR and make the SM PCR and SM RT-PCR methods as useful as the DNA microarray hybridization method. It should be reiterated that we would have missed the opportunity of identifying both the CCBE1 and TCF4 genes as promising candidates if we had not performed SM RT-PCR. The results of DNA microarray hybridization using Illumina's BeadChips could be interpreted that the CCBE1 gene was expressed in all the cell lines and tissues examined, rather than that it not being expressed in some of the cancer cell lines. This is because the values from 24 to 58 were non-zero and negative values were obtained with some other genes as shown in Fig. 2. On the other hand, the value of 2 for the TCF4 gene in primary culture of mammary epithelial cells could be interpreted as no expression and excluded. The results of SM RT-PCR showed high and near linear correlation with the results of real-time qRT-PCR, which demonstrated that the SM RT-PCR results do not require confirmation by real-time qRT-PCR. This is opposite to DNA microarray hybridization where the results always need to be confirmed.

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Title:

Identification of genes on the p-arm of chromosome 8 that exhibit changes in gene expression by the systematic multiplex reverse transcription-PCR (SM RT-PCR) method

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Abbreviated title:

SM RT-PCR of genes on the p-arm of chromosome 8

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ABSTRACT

Losses of the p-arm of chromosome 8 are frequently observed in breast, prostate, and other types of cancers. Using the Systematic Multiplex RT-PCR (SM RT-PCR) method that we developed, we examined the expression of 238 genes located on the p-arm of chromosome 8 in five breast and three prostate human cancer cell lines. We observed frequent decreases in expression of two dozens of genes and increases in expression of several genes on this chromosomal arm. These changes in gene expression of the cell lines were later confirmed by *real-time* qRT-PCR. Additionally and more importantly, we found that some of the changes were also observed in the majority of breast cancer clinical cases that we examined. These included down-regulation of the MYOM2, NP_859074, NP_001034551, NRG1, PHYIP (PHYHIP), Q7Z2R7, SFRP1, and SOX7 genes and up-regulation of the ESCO2, NP_115712 (GINS4), Q6P464, and TOPK (PBK) genes.

Keywords:

Systematic Multiplex RT-PCR (SM RT-PCR), *real-time* qRT-PCR, DNA microarray hybridization, chromosome 8p, chromosomal scanning, gene expression, breast cancer, prostate cancer

1. INTRODUCTION

The activation of oncogenes and the inactivation of tumor suppressor genes both play important roles in carcinogenesis. Most changes in these activating/inactivating processes occur in copy number, gene expression, or nucleotide/amino acid sequences. Therefore, the determination of copy number and gene expression, together with nucleotide sequencing, assists in the identification of oncogenes and tumor suppressor genes. For the activation of an oncogene, a monoallelic dominant change is often sufficient. Examples of monoallelic activation include transcriptional activation of the BCL2 (B-cell leukemia 2) gene by the t(14;18) translocation that places this gene next to an active promoter in follicular lymphoma (1), MYCN gene amplification that is concomitant with an increased gene expression in neuroblastoma (2), and activating mutations in KRAS2 gene in cancers of lung, colon, pancreas, and others (3-5). However, for the inactivation of a tumor suppressor gene, haplo-insufficiency is rare and the disruption of both alleles (biallelic inactivation) is usually necessary.

Quantitative analysis of copy number progressed when the comparative genomic hybridization (CGH) method was invented based on the two-color fluorescence *in situ* hybridization (FISH) (6). In CGH, genomic DNA from a test sample is labeled with one fluorescent color, a reference genomic DNA is labeled with another color, and they are mixed and hybridized with metaphase chromosomal spreads of normal cells. The ratio of the two fluorescence intensities, rather than the absolute intensity, is used to monitor the difference in copy number. Using this technique, many maps of chromosomal alterations in cancer were produced. It was shown that there was a significant degree of heterogeneity among a variety of

tumors, as well as within the same type of tumor. Chromosomal gains and losses, which are indicative of the presence of oncogenes and tumor suppressor genes, respectively, were located on the chromosomes. For example, frequent gains in chromosomal arms 1q, 3q, 8q, 16p, 17q, 20q and losses in 1p, 6q, 8p, 13q, 16q, 17p, 18q, 22q, and X were reported in breast cancer (7, 8). Chromosomal losses were more frequent than gains in prostate cancer and observed with the chromosomal arms 1p, 5q, 6q, 8p, 10q, 13q, 16q, and 18q (9, 10). The use of BAC clone DNA microarrays (11, 12) and cDNA fragment microarrays (13, 14) for the CGH karyotyping analysis of copy number has produced a more powerful and high-resolution arrayCGH method.

Several tumor suppressor genes have been identified in the chromosomal regions of losses. These include CDH1 on 16q22 (15) and PTEN on 10q23 (16). The inactivation of those genes was considered to be the selective force that resulted in the loss of the corresponding chromosomal regions because of the frequent abnormalities and functional failure of the proteins encoded by those genes. Aiming to identify the novel genes with tumor suppressor activity, we started gene expression analysis. We chose the p-arm of chromosome 8 because this arm is one of the chromosomal arms most frequently lost in breast and prostate cancers, strongly suggesting that the region may harbor tumor suppressor genes involved in the pathogenesis of those cancers (8, 17). Although breast and prostate cancers both progress from an early, sex hormone-dependent, organ-confined disease to a highly invasive, hormone-independent, metastatic disease, they arise in two different organs. By pursuing tumor suppressor genes common to these cancers of two different organs, we speculated that the exclusion of inappropriate genes will be easier, whose expression is specific to either of mammary or prostate normal epithelial cells.

Here, we report the results obtained by analysis of gene expression on the chromosomal arm by the moderately high-throughput Systematic Multiplex RT-PCR (SM RT-PCR) method (18-21).

2. MATERIALS & METHODS

2.1 SM RT-PCR experiments to measure expression of the genes on the p-arm of chromosome 8

The following RNA samples were used for the gene expression analysis: a normal and a primary tumor tissue of breast from a patient with invasive ductal carcinoma, a normal and a primary carcinoma tissue of prostate from a patient with prostate cancer, another normal prostate tissue, and a hyperplastic prostate tissue, 5 mammary (BT-20, MCF7, MDA-MB-231, MDA-MB-468, and T-47D) and 3 prostate (DU145, LNCaP, and PC3) cancer cell lines, and primary cultures of normal mammary and prostate epithelial cells. cDNA was prepared by reverse-transcription of total RNA using oligo dT as a primer and the Advantage RT-for-PCR Kit (BD Biosciences-Clontech).

We followed the SM RT-PCR experimental protocols described previously (18-21). Briefly, the genes on 8p were categorized into groups of ~10 genes, and PCR primers were designed to amplify different sizes of DNA fragments from single exons of the genes in a group. After the multiplex reactions using genomic DNA from normal human tissues as a control, the concentrations of the primers were adjusted to produce bands of similar intensities. Once the conditions were elaborated, cDNA samples from the human cells and tissues were then used as templates to examine gene expression. Small aliquots of the SM RT-PCR reaction products were loaded onto an 8% polyacrylamide gel and electrophoresed. The gels were stained with ethidium bromide, the gel pictures were taken, and the images were saved in TIFF format. The band

intensity was measured using the ImageQuant software (Amersham Biosciences) and normalized by adjusting the average band intensities of individual gels.

2.2 DNA microarray hybridization experiments to determine gene expression

For comparison, the DNA microarray hybridization experiments were performed. Illumina's Sentrix Human-6 Expression BeadChips, which contained probes from the entire 23,000 RefSeq collection and an additional 23,000 other expressed sequences, were used. The following RNA samples were analyzed: a normal breast tissue, a normal prostate tissue, primary cultures of normal mammary and prostate epithelial cells, 5 mammary (BT-20, MCF7, MDA-MB-231, MDA-MB-468, and T-47D) and 3 prostate (DU145, LNCaP, and PC3) cancer cell lines. The same preparations of RNA that were used in SM RT-PCR were used in the microarray hybridization experiments. Following Illumina's protocol, biotinylated cRNA was prepared and hybridized with the BeadChips. After washing, the BeadChips were treated with Cy3-labelled streptavidin, washed, dried, and scanned for fluorescence intensity with Illumina's BeadStation 500. Raw data were generated and normalized using the Beadscan 3.0 software. The gene expression data for the genes on the p-arm of chromosome 8 were extracted.

2.3 *Real-time* qRT-PCR experiments to measure gene expression

Real-time qRT-PCR of the selected genes was performed using the same set of cDNA from the cells and tissues that were analyzed by the DNA microarray hybridization experiments, together with the genomic DNA control. The same preparations of cDNA that were used in the SM RT-

PCR were used in the *real-time* qRT-PCR experiments. In addition to this subset of the cDNA samples, additional cDNA samples prepared from 12 matched pairs of normal and tumor breast tissues were also analyzed by *real-time* qRT-PCR. The same primer pairs that were used in the SM RT-PCR experiments were also used in the *real-time* qRT-PCR experiments. The reagent from the Power SYBR Green PCR Master Mix (Applied Biosystems) was used and the yields of the PCR products were monitored using the Mx3000p system (Stratagene) under the default conditions, with the exception that the annealing temperature was raised to 60º C instead of 55º C. Data were analyzed using the MxPro software, and the Ct values were obtained for the individual reactions. The Ct values of the ubiquitously expressed ASAH1 gene, which is located on 8p, were subtracted from those values and normalized.

3. RESULTS

3.1 SM RT-PCR analyses of the genes on the 8p chromosomal arm

We established the SM RT-PCR system consisting of 254 genes. They were categorized into 26 groups. The list of the genes is shown in Table 1, together with the nucleotide sequences and concentrations of the primers used in this study and the sizes of the amplified DNA fragments. We examined the expression of those 254 genes in normal and cancerous breast and prostate cells and tissues. Results are shown in Figure 1. Because the PCR conditions were elaborated so that small amounts of genomic DNA would produce bands, the absence of at least one band was considered to confirm the absence of contaminating genomic DNA in the cDNA specimens. We found that approximately 42% of the genes were abundantly expressed in all of the cells and tissues that were examined. We also observed that approximately 30 genes were not expressed or rarely expressed in either normal or cancerous breast/prostate cells/tissues. The remaining genes were differentially expressed in some of the cDNA samples examined.

Among them, we identified a dozen genes that exhibited unidirectional changes in gene expression in both breast and prostate cancer cell lines. These include the GON1 (GNRH1) (set 16), NRG1 (set 18), PIWL2 (PIWIL2) (set 13), and Q7Z2R7 (sets 16 &18) genes that were found down-regulated in all 5 breast and 3 prostate cancer cell lines and the ESCO2 (set 20), GSHR (GSR) (set 26), NP_115712 (GINS4) (set 23), Q6P464 (CDCA2) (set 16), TOPK (PBK) (set 17) genes that were found up-regulated in all of those cell lines, compared to the expression in normal epithelial cells. We also identified additional genes that exhibited changes in a

majority of either breast, prostate, or both cancer cell lines. Those include CH012 (C8orf12) (set 4), CHO13 (set 5), DEF1 (DEFA1) (set 1), EGR3 (set 20), ENST357748 (ENST000000357748) (set 11), FBX25 (FBX025) (set 1), MYOM2 (set 2), NP_065895 (set 7), NP_859074 (set 19), NP_001034551 (NP_1034551) (set 6), NPM2 (set 19), PHYIP (PHYHIP) (set 14), Q8NEP6 (set 5), Q96KT8 (set 6), SFRP1 (set 13), SOX7 (set 1), TPA (set 24), TR10D (TNFRSF10D) (set 15), and XR_017857 (C8orf48) (set 6) genes. We measured the intensity of the SM RT-PCR bands for quantification. The results were then aligned by the chromosomal locations of the genes and are shown in the left column of Figure 2. To facilitate the comparison, the intensity was shown in gray-scale from black (weakest) to white (strongest). Out of the 254 genes, 238 genes were mapped on the p-arm of chromosome 8 in the newest version of Ensembl (version 43) and the results of those 238 genes are shown.

3.2 DNA microarray hybridization analysis of gene expression of the genes on 8p

In order to compare the results from the SM RT-PCR experiments with the results obtained by an established method of DNA microarray hybridization, we performed the genome-wide gene expression analysis using Illumina's BeadChips. Data for the genes on 8p were extracted and aligned based on the chromosomal locations of the genes. Because the PCR primers for SM RT-PCR were designed based on the sequences that were not alternatively spliced, only the data using the "singular" or "all" probes that detect all the messages from the corresponding genes were extracted from the Illumina data. The average fluorescence signal intensity of >30 beads was extracted and gray-scaled, and are shown, side-by-side with the data from the SM RT-PCR experiments, in the right column of Figure 2. The expression data were obtained for the 230

genes on the p-arm of chromosome 8, 195 of which overlapped with the genes whose expression was determined by SM RT-PCR. Compared to SM RT-PCR, the results from the DNA microarray hybridization experiments exhibited a wider range of intensity as anticipated. There were 25 genes whose messages were not detected (fluorescence intensity below 10 in all the specimens). The number increased to 34 when the cut-off fluorescence intensity was set at 15.

3.3 Gene expression analysis of the selected genes by *real-time* qRT-PCR

We performed *real-time* qRT-PCR to re-examine the expression of the genes that exhibited consistent changes in expression by the SM RT-PCR method. The same set of cells and tissues that were analyzed by DNA microarray hybridization were examined for the expression of the CH012, CH013, DEF1, EGR3, ESCO2, FBX25, GON1, GSHR, MYOM2, NP_065895, NP_115712, NP_859074, NP_001034551, NPM2, NRG1, PHYIP, PIWL2, Q6P464, Q7Z2R7, Q8NEP6, Q96KT8, SFRP1, SOX7, TOPK, TPA, TR10D, and XR_017857 genes. Because of high expression of the messages in all the samples in both the SM RT-PCR and the DNA microarray hybridization experiments, we selected, as a control gene, the ASAH1 gene (22). This gene encodes N-acylsphingosine amidohydrolase, also called acid ceramidase (AC; EC 3.5.1.23), which catalyzes the synthesis and degradation of ceramide. The Ct values obtained by *real-time* qRT-PCR were plotted on the X-axis. The $\log_2$ values calculated of the measured band intensity and fluorescence intensity of the genes from the corresponding cDNA samples were plotted on the Y-axis. Results from all the selected important genes are shown in Figure 3. The results are shown on the same scale, and the result of the ASAH1 gene was also enlarged and shown next to the original figure on the top row. A higher degree of linearity was observed

between the results from the SM RT-PCR experiments and the results from the *real-time* qRT-PCR experiments than between the DNA microarray hybridization experiments and the *real-time* qRT-PCR experiments. This is reasonable considering that both the SM RT-PCR and *real-time* qRT-PCR are PCR-based and the same primers were used in those experiments. The differences observed by SM RT-PCR were confirmed to be real by *real-time* qRT-PCR, although some of them were not observed by DNA microarray hybridization.

We next examined whether the same differences in the expression level that were observed in the breast and prostate cancer cell lines were also present in the clinical specimens of cancer. Because we had matched normal and tumor pairs of breast tissues from a dozen breast cancer patients, we analyzed the expression of the selected genes in breast cancer by *real-time* qRT-PCR. To normalize the Ct values of the individual specimens, we subtracted the Ct values of the ubiquitously expressed ASAH1 gene from those values. Down-regulation was observed with the following genes in a majority of 12 breast cancer cases: MYOM2, PHYIP, SOX7 (10 cases), DEF1, FBX25, NP_001034551 (9 cases), CH012, GON1, NP_859074, NRG1, PIWL2, Q7Z2R7, SFRP1 (8 cases), Q8NEP6, Q96KT8, and XR_017857 (7 cases). Similarly, up-regulation was observed in a majority of breast cancer cases with the following genes: TOPK (10 cases), Q6P464 (9 cases), ESCO2, NP_115712 (8 cases), GSHR (7 cases). For the remaining genes, down-regulation was observed in 4 (NP_065895), 5 (TPA), or 6 cases (CH013, EGR3, NPM2, TR10D). Many of the important results are shown in Figure 4, by plotting the subtractive Ct values of the tumor tissues on the Y-axis and those values of the normal adjacent tissues on the X-axis.

4. DISCUSSION

Previously we established the SM RT-PCR systems of families of glycosyltransferases (18), HOX homeoproteins (19), and integrins (20). We also gradually increased the size of coverage from a few dozen genes (21) to more than a hundred genes in a few cytobands (23). Here we attempted to establish the SM RT-PCR system of more than 200 genes on the entire arm of a chromosome. Excluding the DNA microarray hybridization approach, this SM RT-PCR analysis is one of the largest attempts to understand the expression of the genes on a chromosomal arm-wide scale. We aimed to incorporate as many genes as possible into the system. Because the defensin genes were highly homologous one another and possessed short coding sequences, we were unable to design primers for several members of the defensin gene family that selectively amplified single species of the gene members. Nonetheless, we included 254 genes in 26 multiplex reactions as shown in the primer list in Table 1 and the genomic DNA lanes (G) in Figure 1. However, the Ensembl database was not finished at the time that we retrieved the gene and sequence information. When we aligned our results in the most recent version, 43, we found that 11 genes that were previously mapped in the region did not exist any longer. Furthermore, a few dozen additional genes that were not previously mapped have been added. These include novel protein-coding genes, pseudogenes, miRNA genes, snRNA genes, and snoRNA genes. Because of this addition, Figure 2 has many open spaces, for which no expression data were available. 195 of the 238 genes whose expression was determined by SM RT-PCR overlapped with the genes whose expression was determined by the DNA microarray hybridization experiments. A generally good correlation was observed in the results between the SM RT-PCR and DNA microarray hybridization experiments, except that the expression of approximately 40

genes were detected only by SM RT-PCR. We think that the probes of those genes used in the DNA microarray hybridization were either inappropriate or not functioning as expected. The results shown in Figure 3 also illustrate this problem. To calculate the $\log_2$ values of fluorescence intensity from the DNA microarray hybridization results, we used 0.1 for the values below this number. Still, when the fluorescence signal was weak, as in the cases of NP_001034551 and PHYIP, no correlation was observed between *real-time* qRT-PCR/SM RT-PCR and DNA microarray hybridization. However, when fluorescence signal was strong, both SM RT-PCR and DNA microarray hybridization exhibited linear correlation with *real-time* qRT-PCR as shown with the GSHR, SFRP1, and TOPK genes.

In addition to the cell lines, we also examined the expression of the selected genes in the clinical specimens of breast cancer. As opposed to the *in vitro* cultured cancer cell lines that consist of a relatively uniform population of cells, tissues are made of several different types of cells and their ratios vary among different specimens. Therefore, measurement of the Ct values without standardization was not informative. We used the expression of highly and ubiquitously expressed ASAH1 gene as a standard. By subtracting the Ct values of the ASAH1 gene, we compared the relative ratios of the gene messages among different specimens. Rather than comparing the normal and cancer tissue specimens as two groups, we plotted the results from the individual pairs of cancer tissue specimens and their corresponding normal adjacent tissue specimens on the Y- and X-axes, respectively. The tendencies of up- and down-regulation in gene expression were easily confirmed with most of the genes examined. For several genes, the tendencies were not clear with the breast clinical specimens. Several potential reasons can be speculated. One possibility is that the cancer cell lines may have acquired down-regulation in

expression of those genes after they were brought into *in vitro* culture. Another possibility is that cells, other than cancer cells, that were present in the tumor tissues express these genes and losses/decreases in cancer cells may have been masked.

Among the genes that exhibited a matched tendency of up-regulation in the breast tumor tissues and breast cancer cell line cells, the tendency was striking with the ESCO2, TOPK, NP_115712, and Q6P464 genes. Because the ESCO2 gene is required for the establishment of sister chromatid cohesion during S phase of cell cycle (24), the TOPK gene encodes serine/threonine kinase that binds to the PDZ2 domain of Drosophila Discs-large (Dlg) tumor suppressor protein that regulates the cell cycle and/or cellular proliferation (25), the NP_115712 protein is a component of the GINS complex that is essential for the initiation of DNA replication (26), and the Q6P464 gene is associated with cell division cycle, up-regulation of these genes in tumors and cancer cell lines may simply be a reflection of a higher number of dividing cells in those specimens. These four genes were elevated in gene expression in all 5 breast and 3 prostate cancer cell lines that were examined, suggesting that this is a likely possibility.

Among the genes that were down-regulated in a majority of breast cancer cases, 4 genes, GON1, NRG1, PIWL2, and Q7Z2R7, were also down-regulated in all 5 breast and 3 prostate cancer cell lines that were examined. Additionally, 4 genes (NP_859074, NP_001034551, PHYIP, and SOX7) exhibited down-regulation in all the 5 breast cancer cell lines examined and 1 gene (SFRP1) exhibited down-regulation in a majority of breast and prostate cancer cell lines. Two genes, CH012 and XR_017857, exhibited down-regulation in a minority of cell lines, and 5

genes (DEF1, FBX25, MYOM2, Q8NEP6, and Q96KT8) showed decreased expression only in the breast cancer cell lines. Among these down-regulated genes, statistically significant tendencies were observed with the MYOM2, NP_859074, NP_001034551, NRG1, PHYIP, Q7Z2R7, SFRP1, and SOX7 genes, shown in Figure 4. The tumor-suppressing role has been well established of the NRG1 gene. The gene encodes neuregulin 1 (heregulin) that interacts with the NEU/ERBB2 receptor tyrosine kinase to increase its phosphorylation on tyrosine residues (27). The purified protein induces phenotypic differentiation of breast and prostate cancer cells and inhibits cell growth (28, 29). The SFRP1 and SOX7 genes play a similar role in carcinogenesis by repressing the Wnt signaling inside the cell. The SFRP1 gene encodes a secreted apoptosis-related protein that interferes with the Wnt-frizzled signaling pathway (30, 31). The potential role of the SFRP1 gene in tumor suppression of breasts and prostates was previously suggested (32, 33). The SOX7 gene encodes a transcription factor that possesses a functional transactivation domain in the C-terminus and significantly reduces Wnt/beta-catenin-stimulated transcription (34). The identification of three genes, NRG1, SFRP1, and SOX7, which are known to be involved in carcinogenesis among the candidates, indicates that the approach is working as expected.

In addition to those cancer-related genes, we also identified MYOM2, PHYIP, and three poorly characterized candidate genes. The MYOM2 and PHYIP genes encode myomesin 2, an M-band protein of sarcomeres (35), and phytanoyl-CoA hydroxylase-interacting protein (36), respectively. The NP_859074 gene predicts to encode a protein with the EF-hand domain (37), and the NP_001034551 and Q7Z2R7 genes predict proteins of 121 and 83 amino acid residues, respectively. Little else is known of those genes, however, the Q7Z2R7 gene is separated from

the NRG1 gene by only 1,577 bp and that the expression profiles of those genes were similar in the cells and tissues that were examined. Because the orientations of these genes are the same, there is a possibility that the Q7Z2R7 sequence may be transcribed run-off in the 3' untranslated region of the NRG1 gene messages rather than transcribed independently from its own promoter. Further studies will be needed before concluding that these candidates are genes with tumor suppressor activity. In summary, we have shown that the SM RT-PCR approach is successful in the identification of genes with altered expression through scanning of the genes at the subchromosomal level. It should be emphasized that by performing multiplex reactions of 10 genes on average, the number of reactions was reduced by 10 times in the SM RT-PCR experiments, as compared with *real-time* qRT-PCR of individual genes. Together with more flexibility in designing the SM RT-PCR system than DNA microarrays and using pre-confirmed primers, this advantage may allow the SM RT-PCR find its niche between the discovery method of high-throughput DNA microarray hybridization and quantitative *real-time* qRT-PCR.

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FIGURE LEGENDS

Figure 1. SM RT-PCR results of breast and prostate cells and tissues

The results of the SM RT-PCR experiments are shown. There are a total of 26 sets. SM RT-PCR was performed to examine gene expression changes in breast and prostate cancer cells and tissues. The sources of cDNA are abbreviated: a normal sample (NB) and primary tumor (TB) of breast tissue from an individual; a normal sample (NP), and primary tumor tissues (TP) of prostate from an individual; a normal prostate tissue (NP) from a third individual; a hyperplastic prostate tissue (HyP) from a fourth individual; primary cultures of normal mammary (MP) and prostate (PP) epithelial cells; and MCF-7 (MCF), MDA-MB-468 (468), MDA-MB-231 (231), BT-20 (BT), T-47D (T47), PC3 (PC), DU145 (DU), and LNCaP (LN) cancer cell line cells. The locations of the DNA fragments amplified from the individual genes are also shown at the left side of the gel pictures. The symbol M denotes DNA fragment size markers, and the symbol G shows the results of genomic DNA control.

Figure 2. Intensities of the bands amplified by SM RT-PCR and the intensities of fluorescence detected after DNA microarray hybridization of the genes on the p-arm of chromosome 8

Data that were obtained by the SM RT-PCR experiments that are shown in Figure 1 were used to prepare this table by the densitometry measurement of band intensity. In order to normalize the values, the average band intensities of individual gels were adjusted. The partial results of the

SM RT-PCR experiments are shown in the left column. The values of the band intensities of the PCR-amplified fragments were aligned by their chromosomal locations, and are shown in gray scale, with white as the strongest and black as the weakest. Data on fluorescence signal intensity were extracted for the genes on 8p from the DNA microarray hybridization results, normalized, and aligned. Results are shown in the right column. The gene names, cytobands, the starts and ends of the gene locations, and the primer sets, are also shown.

Figure 3. Correlation between the band intensity obtained from the SM RT-PCR or fluorescence intensity obtained from DNA microarray hybridization and the Ct values obtained from the *real-time* qRT-PCR experiments

The $\log_2$ values of the band intensity and fluorescence intensity were calculated and plotted along the Y-axis with black diamonds and gray squares, respectively, against the Ct values on the X-axis. The ASAH1 gene was used as a control, because this gene was ubiquitously expressed in large quantity in all the cells and tissues that were examined in both the SM RT-PCR and the DNA microarray hybridization experiments. Negative and zero values obtained by microarray hybridization experiments were assigned the value of 0.1 for these graphs. The portion of the ASAH1 results was enlarged and is also shown on the top row.

Figure 4. The expression of the selected genes in matched normal and cancer breast tissues

The gene expression was determined for the selected genes by *real-time* qRT-PCR using cDNA prepared from 12 matched normal and cancer breast tissues. The results from the genes that

showed consistent and meaningful changes in gene expression in both the cell lines and clinical specimens are shown. In order to normalize the expression data, we used the expression of the ASAH1 gene as a control. The subtractive Ct values (minus Ct ASAH1) of normal tissues are mapped on the X-axis, whereas those of the corresponding tumor tissues from the same individuals are on the Y-axis. The line y=x is also shown. The dots above the line indicate down-regulation in tumor, whereas dots below indicate up-regulation.

Table 1. Primers used in the study

Set	Abbreviated Gene Name†	Frag. Size (bp)	Primer 1 Sequence	Primer 2 Sequence	Final Concn (nM)
1					
	ARHGA	144	TGAGAAGCAAAGCACGCCGGGC	TCTGCCAGACCATGACGGTCG	94
	SOX7	108	CATGGATCGCAATGAATTCGACCA	GGTGTCACCTGGGAGACCGGAAC	94
	FBX25	95	CTCAGGACACCCCTGCACGGC	GCAGCCCTTAAAACTTGAAGAGGTCGAT	141
	NP_078883	86	TCGACATCAGCTTGCCCGAGAA	CCAGTACGTCTGTCCATTGCACTCGTAG	469
	NP_079043	81	TGCCAGAATGGTACACAAAATCTTTTGG	TCTGGTTACTGAAGGAATCCCGGATCT	94
	CSMD1	77	CCAAAAGTTCAATACAATGGCTATGCTGG	TCATACATGGGGTTTTCAAACGATGC	188
	MTMR9	72	AAGCAAAAGTCAATATCCTTCGAAGGCAGT	AGGGACTCTCCTGCATCCCGTC	94
	DEF1	68	GGCCTGCTATTGCAGAATACCAGCG	CCTGGTAGATGCAGGTTCCATAGCGAC	75
2					
	TNKS1	120	CAAATGCTCTTCTGTAGAGTGACCCTTGG	CAGCCCATTGACGCTCGGTCTAC	94
	NP_919260	109	CCCAAGGCCTACACCAACTCGG	AGTCGCAGGGCAGCGAGCTGT	469
	MYOM2	101	CCGAAGTGATTTGGTTCAAGAACGACC	GCCTTTGATGGTCATGCTGACGTACT	75
	PINX1	94	AGGCCCCTGCTGGGACCAGAGTT	GGGCTTCAGGGTGAAGTCCCG	94
	DLGP2	88	TCCCTGGACCTGCCCGACAGAC	CGGAATTCTGCCGGAAGGACG	141
	BLK	82	GAGCTGTACCGCGGCGTCATC	GCACCGACTGCAGGAACTCGA	469
	D103A	78	ATTGCAGAGTCAGAGGCGGCCG	GCGTCGAGCACTTGCCGATCT	19
	MCPH1	74	TGTGAACTAGTCCACCTGTGCGGAG	TAGGGCCCGATGACGATGCTG	141
3					
	NP_940866	155	AGAACACCCCAGGGATATACACCTCG	TGGCTCTGGGACTCCCGAGACT	54
	C8orf5‡	119	TGTCTTGGGGTATCAGATTTACAGCGTAACA	CATTTTCCTGAAAATCTGCTGCAGATTTAAG	214
	Q96LV3	103	GGGGATTAAGTGGAGCTTATGGACTGC	TTTCAAACCCCACCCAAAATTCACTC	107
	CH014	94	GGTGGGAGGAGAGCCCGAGATC	CCCAGGTGAGACTTGCAAGTTCACG	161
	ENST297485	87	TGGCCATCTGCAAGATATGCCG	GGACATTTCCTTGGAGCTGCTCGAG	64
	NP_1027009	82	GAAGCTGGAGCACAAATGTCCCG	TTCCACTTCCCAAGCTAAGCCTCG	43
	Q8NF75	77	CACAGGGTGTTATTCCCATCTCATCG	AAGTCAGCCTCAGGATGCGGGT	536
4					
	Q8IWN7-2	170	GCACCTCCTCCTCCCGGAAGAGT	CAACTGCTTCACAGGAAAGCGCA	75

XKR5	157	CCCGACACCATGGCCGACATT	CACCAACAGCTGCATGGTGACTTAGC	75
NP_778250	146	GGATGACGCCAAGGGCTCGAC	ACTCCGCCTCCTCCGGGCTAT	75
C8orf15‡	135	AGCAGGTAGCACTGGAGCCGATC	GCAGTTCCAGTGTCCCGTGGTC	75
CH012	82	CAGAAGTACAAGGTGAAGAATGCATACCGA	CCAAAGAAGCAAAAGTGCTAGCACCA	125
NP_689484	78	GCCCCTAATGGACCTGCATGGT	CAAAACCGACAGCTGGTATCGTGG	125
5				
NP_1035121	172	GGAGCTGCTGCAGCGCCAGAT	CTTCGCTTTCTTTCCTTTGCTCGC	107
Q86YV5	152	ACCCCATCAAGCGTATCCGCA	CAGGGCCCGCTTCATGTCGAT	536
CH013	132	TGCTCAACGATGCCACCTACGA	TGATGTTCATCTTGGTCACGCCAATA	161
XKR6	120	TGACATGCCAAGAAAGCGATACCC	TCTCGATATCGAATGCCTACTGCGG	107
Q8NEP6‡	101	CAAATATGGATTTGGGGTGTGCGTAA	TGTCCTGCAGCCGCACCGATA	214
Q8N852	91	TGGGCTTCCTGGGACTCGGTG	GCTTGGAAAACCACAAAGAATGACGC	64
Q8TCU9‡	85	GAAGCCAGGGGAACAAGGTTAAAAGG	TGTCACCATCTTCAGCCAAGCCAC	107
6				
MSRE	158	GAGCAACATGGAGAAGAGAATCCAGC	TCCCATGTCCCTGGACTGAGGAAA	102
XR_017857	145	GCCCTGTCTGCCTTTCTGAAACAA	GGTCATCTGAAAGCCTGGGAAAGT	102
649548	124	AGCCACAAGTGCTGCTGATGTGTA	GGATTGTTTACCCTGATGGCCAGA	68
FDFT	112	CCGGAGAATATTGACTTGGCCGT	ACACACTCTGGTTTCTGAGTCTCG	68
730602	103	GAATATTGGATGGATCCTGAAGGCGA	CAATGACAGAAATCTGTTCCTTCAGCTGGC	68
Q96LV6	90	TGTTTGTGCCTAGCACGATTGGG	AGATGGTCCTGGGTCTCTGAATAC	102
NEIL2	84	AGAAGTTCCATCGAGGACAAGCCT	AGAAGTATCTCTGGTCCAGCAGTGT	102
Q96KT8	78	AAGCTTGTCTTTGCCTTCACGCC	CCCGGACCTCTTCTGATAAGGAAT	205
SGCZ	74	ACAGTGTATGAACTCTGCGTCTGC	TGACAAGTGGAACCTACTCCTGCT	102
NP_1034551	71	GCCTGTGCTTCCTTCAGAGACTCA	GGAGTGAAATGAAATAGTGCGGTCC	205
TUSC3	68	TCAGACCACCCAACTACTCTGGTA	AAGCAAACCTCCAACAAGCGACAC	102
7				
NP_1027009	138	GCCACTTTACACTGTTGCTCCCAT	AAATGCCACTGCCACTGCTATCTG	136
GATA4	125	TCTCAGAAGGCAGAGAGTGTGTCA	CCGGTTGATGCCGTTCATCTTGT	102
BLK	114	GGATGGTCTATGCCAGAGGCTGA	AGTTTCCTGACCAGCCTGAGAGA	102
CH013	105	CACGTGGAAGAAGTGGGTGCAGAAG	GGTCTCCAGCCTCAGTTTCTCGGT	55
Q96LV6	97	TGTGGCTGTTGGACGCCTGTC	CAGAGAAAGACGCAGAGTGGAAGT	205
NP_065895	90	TCCACAGCCTTTATGCGCTACTAC	CAGGATACGGAGCTCTGACACATTC	136
CATB	78	AACACGTCACCGGAGAGATGATGG	AGTAGGGTGTGCCATTCTCCAC	68
NEIL2	74	CAGAAGGGCCGTTGGTGAGGAAAT	CCCTGTCTTGACCACCTGCTGA	68
CH014	71	CCCTGAAAGTAGCAGGACAGCCTTA	CCTGGACTCACAGACTAGACTCTTGC	68

	BLK	68	ACAAGCATTTCGTGGTGGCTCTGT	CTTCAGCATCTGCAGGTCCCGATCATT	68
	CH012	65	AGTGGAGGCCGGTAGTGCTGAAT	TGAGAGAGGCAGGCGGAAGTCTTT	68
8					
	Q86YV5	100	GGAAGAGGACCATCGGACGATCTA	TTCACCTACCTCGGAGTTCTGGCT	188
	NP_004216	91	CAGTGTCCAGATCAACAGCCATGT	ACTCACAACCACAGGAACTTTCCCTC	75
	THEX1	83	GCCTCACTGTGGTCTTGATGACTCTA	TGATTCGGAGTTCACACCCATCCT	125
	NP_078883	70	TGCGCCAAGAGAGGTTTGCCTTTA	AGCTGAATACAAGGCCTCAGTGGT	75
	MSRA	65	ACATCCGGGAGGGACAGACTTTCTA	GTTCTTGCTCAGGTACTGCTGGT	75
	NP_919260	61	TGATGACGCTGGGCATGGTGTT	AGGGTGCCGGTCAGGTTGA	313
9					
	PNMA2	178	TCATGCACATAGTGCAGGCAGACA	AGCAGGGTTTCTAGCCGTAACAC	167
	LOXL2	163	ACTCCTGATATGCCGTACTTGGACC	GGGAAGTCTGGGTCTCGTTTGTTT	67
	NP_060561	149	TGGTGGCTGGGAAACATTCTTGTC	TCCCATACACATGCAGCTCTCGTA	83
	ADEC1	136	CCCAAAGGATTTCAGTACATCTTGCCG	TTCTAGAAGGTGGTTCCCACACAC	83
	WRN	124	AATTGGCATGCACTTATCCCAAGCGG	TGAGTTGACGGGAGGGTTTCGGAT	167
	FGL1	113	GTGTCACTCTGCAAACCTGAATGG	CAGATTTCAGAGAATACCACCACCCA	125
	VATB2	103	ACATTGGCTGGCAGCTACTCCGAAT	TAATGCTTTGCAGAGTCTCGAGGG	83
	D104A	86	TATGTGGTTATGGGACTGCCCGTT	GCATGCATAGGTGTTGGGACATCTTC	83
	IKKB	73	CATGAATGCCTCTCGACTTAGCCA	AGGTAAGCTGTTGGAGGCCGT	167
10					
	TEX15	195	GACCACAGTTGCAAGTACTGCCC	GCTTGTGGTAATGGCTGATGAGAAGC	41
	MCPH1	179	GTTGTAGACAGGCTGGGAAAGAAGAC	GGAAGTGGTACCTTTGTTCTGTGTGC	68
	2ABA	164	AGAAACACAAAGCGAGACATAACCCT	TGCCAGGCTGTGTGAAGGATTT	136
	ADAM2	137	CATTTACCATTCCAAACCAATGAGATGGC	CGCTTGAATAGTCCTCAGTTCTCCAT	136
	STMN4	125	AGCAGAGAAACGGGAACATGAGAGAG	CCTCCCTGTTCTCCTTGTTGGATT	102
	TMM66	114	ACACCCTTCTCAGACTCGTGGTACT	TGAACATACCGAATAGCTGCCCGA	68
	ADA28	104	TTTGCCCTGGAAAGGACGGATAGT	CACACTTAGTTCCATTGGCCACCA	102
	UBXD6	95	TCTACTTCCTTTCCCAGACGGCCT	TCCTCCAGGATGAGTACAGTGTCCA	102
	PGFRL	87	TCCCAGTGGCCCTCCCTCAACAA	AGGACAGTGCAGAGCACACTGAT	102
	ASAH1	80	AGCCTACTTTATCCTGGGAGGCAA	ATACATCCAATGATTCCTTTCTGTCTCGTG	170
	NM_199205	69	TGATGCCAACTGGTGTGGCTACTT	CGGATCCCAAGGGTAAGGAGGAT	205
11					
	LZTS1	182	AGGAGAAGGAGAAGGTGATTCAGTACC	AGTGGCTATGATGTCCTCGTAGGG	125
	Q8N1G8	167	TACGGCCACCTTGTATCTTGTCAC	GGAGCACAGAGAGCTAGCATCAGG	63
	ARHGA	153	GACCTGTCCTCCTCATCTGGGTCC	CGAGCTGGCCTTGGCTTTCTTGGC	63

	LIPL	140	GTCTTACACACATTCACCAGAGGGTC	TCTGCAATCACGCGGATAGCTTCT	250
	Q8N8I7‡	128	GGAGGAGATGTTGTGTGCATGTGAGA	TCTGTCTGCGTGAGCACATTCGCATA	125
	BMP1	117	AGGTGTACTCGGCGGGAGATTCTGT	TGCTGTGGAGTGTGTCCTGGAACTT	63
	Q16016	107	AGGCCTAATCGCCACTCATCAGCAAA	TCGGTTTGTTCAGAGGTAATGGAGGG	94
	ENST356349‡	98	AAGAGACTAGTGGCATGTTGTCCC	TGCTTAAAGATTTCTTCCAACAGATAGCCA	313
	Q9UDD8	90	TAGAAGGCATGCTCCTGACTGTGA	GGTGAGGATTCACGATGATTGGGT	63
	ENST357748‡	77	GAAGCTCACTGGTGTGTGTTCCTCA	AAACATGGTGGCCAGCAGCATTTG	93
	ENST338711	72	AAAGCATGGCCATCATCTCCCACA	ATTCTGCAATGTAGAGGGCGGCA	125
	CLN8	68	GTCAGCAGCCTGTATCTGCCTCATTT	ATGATTAGCGTAAGCAGAGCCAGTCC	188
12					
	NP_079091	216	GCTGCTGCAGGAGTGTCTGA	TGGCTTTGCAGGTCTTCCTCCAT	156
	PP2BC	168	AGGGTTCTCGCTTCAGCACAAGAT	TTCTTCCCTTGGTCGCTCCTGT	156
	Q86YR2	141	AGTCCCTATGCAAGTAGCCCAGTT	GGAGGGAGGAATGATGATAAACCAGG	94
	EFA6R	129	CAGCATTCTCAAGGAAGGAGGCAA	CGCTTGACTTTGGCAGTGATTGGA	94
	Q7L3Y3	118	CCCTGCAGAGAGTGGTGATAACACAT	GAAGTGGCATTTGAAACCCAGGCT	94
	GFRA2	99	TCATCCCAGGGAGTAACAAGGTGA	GTTTCAGCATCAGGACAGACAGCA	250
	MTMR7	91	CCGACAGTCAGTTACAGATTACCTAATGGC	ATGTCTTACTTCTTCCAGGGCCTC	219
	Q8NB85	84	TGTCCTGTGAAACCCTGTTACCTC	AGTAGGAGGTGACTCCTTATGGGCA	188
	SH24A	78	GAGGAACCCATCACTTCCCTGG	CAGGTAGTCAGGCAGCTGGT	250
	ZDHC2	73	TGCAAAGCCATTGAGAGAGTCCCA	GTTTATGCTGCTCTCCGTCCAAGA	188
	SPG11	65	AGGCTCTCGGAGAACTCAGGGAAA	GCGTAGCAGCTGAAACCCGTTTGT	156
	ENST360191‡	61	AGGAAGGCTCTCCTCCACATGTTT	TTCCATTCGGCAGCGGCTTCTCTG	94
13					
	FGF17	217	TGTTCACGGAGATCGTGCTGGAGAA	GAGCCCACAAACTCGAACTGCTT	115
	Q9NUU8‡	200	CACTGCCCACCGCCCACCTCA	GCTGCAGCACAAGATCCTGGAGACT	58
	SFRP1	169	CACCAGCTGGACAACCTCAGCCAC	ACTTAAACACGGACTGAAAGGTGGG	58
	PIWL2	142	CTGTGCCACATGTACTGGAATTGGCCT	ACAGGAAGAACAGGTTCTCGCACA	58
	DOK2	130	ATATACGATGAGCCCGAGGGAGT	CTGGCTGGACATGCTGGAGG	87
	CGAT1	119	ATGAGAAGCGCTGCATGGACGA	TATCTCGTGCCTGAACACCAGCAT	87
	Q8NEE2‡	109	ACAAAGACACCCGGAACCACCACA	AGGGACCATATCCGGGAGAGC	288
	CTR2	100	ACAATGAAGAAGATGCTTATCCAGACAACG	GGTGAACTGAGATTTCTTGGGTGATGG	115
	Q71JB5	79	TGCTGGAGGAGTTCTGCAAGGA	GAGACGTTCTGGCGAGGATCATGT	87
	VMAT1	74	TATGCAACCCAGAAGCCCACGAA	TACTCCTCATGGTCAGGCTCCTCAT	87
	DEMA	70	TCTCAAGGGTATTTGCCATGTCCC	TTGAGCTCATTCCGCTTCCACAGA	115
	ENST319916‡	66	TTCCTGTCCATGTGCCTGGTCACT	CTCAGGGCTGACAGACAGGAGGAT	115

Q8WUT8	62	CCGACATGGTGGATAAGAACAAGTGC	CACCGCTGAAGTGAATGACATGTTGC	115
14				
PHYIP	201	TGCATGTACACGGCCTACCACTA	GTCGACGGGCTCAGTGTAGATGAT	80
GFRA2	170	AACGCCATCCAGGCCTTTGGCAAC	TGGACAGACGTGCAGGTGGTGATGA	107
Q9P1G9	156	CAAGCCAGGGTTGGAAGAACCAAA	TGAGTCAATGGCCTTCACCTCCAT	80
LGI3	143	GGGACGACAGAAGTTTGTACGGTT	CCACCACAATGTGTCTATACACCAGC	80
PDLI2	131	CAGGCTGTGCGCATCCAGGAG	CGGGCATGCTTCTCACAGTACA	80
INT10	120	CACCACACTGTAACTCGAGGCAT	AATGCAGAACCTGTGCAGAACCAC	107
BIN3	110	ACCTGTCCCATCAGCTTGACCA	TCAGTCATCGGCCACAATGGAGA	107
Q96BB3	93	GGAAGGGCAGCATCTTGATTCCAT	GTCCTGAATACATGGTGAGGACCAC	107
XPO7	86	ACAGTATTGTGAACAGCCAGCCAC	ATTTCGCTCGATGCCTTCCATCAG	107
PSPC	80	TGGAATGCTCTCTGCAGGCCAA	TGCTGAGCCTGCATCTCGCC	134
NP_1013864	75	ACCTCCCTGAAAGGCCTCAGT	CTCGTTGCTTCTTAACCACTAATGATGGCA	268
RPO3D	71	CTGGGACACGTGAAGCACAAACTT	TACCGGTGTTTGTGATCCAAGAGGGA	107
BD02	67	ACCTGCCTTAAGAGTGGAGCCATA	TGCCAATTTGTTTATACCTTCTAGGGCA	80
ENST357748‡	62	AGCCTACACCATAATCTCAGGATCCACG	TGAGGAACACACACCAGTGAGCTT	161
15				
NKX31	193	CTCACGGAGACCCAAGTGAAGATA	CCACGCAGTACAGGTATGGGTAGTAA	125
TR10B	162	TAAAGGTGGCTAAAGCTGAGGCAG	AGTGGTCCTCAATCTTCTGCTTGG	63
STC1	148	CAGACAGACCACTGTGCCCAAACA	CACTCTCATGGGATGTGCGTTTGA	94
CHMP7	135	GACATCCTCCTTCAGGATACCACCAA	GGACAGTTTCTCAAGTTCAGCTTC	94
Q9Y3T6	112	AACTCCTGCGTCTGGTGAAGG	CGGACAGCAGGCCGCTCTTTCTTT	94
NFL	102	AAGAAGAAGGAGGTGAAGGTGAAG	TCTTCTTAGCTGCTTGTTCCTCCC	156
TR10D	93	CACTGGAAGAAGGACATGCAAAGGA	TAGCAGAGCCTGCCTCATCTTCTT	125
MFRN1	85	TCCAGTCCATCCACTTCATCACCT	ATGATGTGGGACTGCGGGTTGTAG	94
TR10A	78	CCTGCCTCTTCTCATTACCTCTCA	TCAAACAAACACAATCAGAAGCACA	94
ADAM7	72	CACCATCTTGGTTGTTGTGCTTGTCC	CGGTAACGAACTAATAGTATAAGAACTCCG	188
PEBPL	67	TTCCACCTGGGCGAACCTGAA	GGGTTGGTGAGTCCTGGTAGTTCT	125
LOXL2	63	GGTTCCTTCAGCGAAGAGACGGAAA	CTGGTTGTTTAAGAGCCCGCTGAAGT	125
16				
SCAR3	191	TCAAAGGGCAGCTTTGGAACTGGA	ATCCCTGGTTCACCCTTAGGC	94
Q6P464	160	CCTGCTGCTGGTTCTTCCGAT	CAATCCTTTCCAAGGCAGTCAGAGAG	125
TRI35	146	CGCCACTGCCACCTGTACACCTTC	CAGCCATCCAGTTCTTCCTTGACA	125
ENST355177	133	TCATCAGAAAGTACCACAGACTGGGC	TGCAAGCCAAATAGAGAGGCCTCAGA	63
BNI3L	121	GAGTTCCACTTCAGACACCCTAAACG	GGAAGAGAGATGGAATGAACACCTTCAG	188

	CLUS	110	AGCTCTTTGACTCTGATCCCATCAC	TTTGCGGTATTCCTGCAGCGCTTT	125
	FAK2	91	AGATGCTGACGGCTTCACACA	TGGGCCAGATTGGCCAGAACCTT	125
	ADA1A	83	AGGTCTGCTGCTGTGTAGGG	GGAGATGGTGTGGACCTTAATGGTTG	94
	Q7Z2R7	76	GGTCCTCTGTGTAGGTGAATGTGTCC	CACCTCTAACAAGACAGTCACACTGAAA	94
	HMBX1	70	AGCAATCCTGGAGAGTCATGGGAT	GTCGACATCATCACTGTTTGAGTGGC	94
	KCTD9	65	GTGATCTGTCTGGGTGTGATCTTCA	TATAGCTCCCTTCACGTTGGACCCT	94
	GON1	61	GTGCGTGGAAGGCTGCTCCA	TCTCTTTCCTCCAGGGCGCAGTCCATA	125
17					
	DUS4	176	TTAAGCAGCGCCGCAGCATCATC	AAAGCTGAAGACGAACTGCGAGGT	75
	Q96T53	147	CAAGAAAGTGGAACCAAAGCACAGC	CCCAGCAAACGAAACCAAACACCT	113
	NP_060720	122	TATTAAGGTGGAGGACACAGCCAAGG	GCACTCTGAGCATCTCGTCATTGT	150
	TOPK	111	GGCCACCTATTAATATGGAAGAACTGGATG	CAATGTGTGCAGCAGAAGGACGAT	60
	Q6ZP73	101	TCGGGAGTGAATACAAGGAGATGGAG	CCTTAGGAGGAATTCTTGCATGCCCT	150
	DCTN6	92	TTGACAAGTGGCTGCATCATTGGG	AGGCAGTCTGCACCATAGATCACC	75
	COE2	84	ACCGTTGGGTCTTCCAGCACAT	GGCAAAGGCACTCTTCTGTTTGAC	113
	HYES	71	ATCCTGATTCCGGCCCTGATGGT	ATGTGCTGGGACATCTGAGGAAC	150
	PNOC	66	AATACTTGGTCCTGAGCATGCAGTCC	ACACATTACCATTCTGGTGCAGGG	225
	DPYL2	62	GCACCACCCAGCGTATCGTG	CTAGCCCAGGCTGGTGATGTT	300
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	NP_1015508	177	CTGAAGGTAAGTGAGGTGAGACCAC	GCATTCTTGTTCTTCACCACTGGC	144
	NP_076930	148	ACTGGCCTACCTCATGCTGTACCA	TCATGCTTCCAGACCCTGCC	231
	UNC5D	135	TATTTCGCTACACAAAGTAGCCCATCTGC	GTTTGAGAGTTTCGTGTGTGTCCTCC	144
	EXTL3	123	GCATCAACTTCTTCGTGAAGGTGTACGG	AGATGAACTTGAAGCACTTGGTCTTGTC	144
	WRN	112	TGCACTTATCCCAAGCGGTGAAAG	TTGACGGGAGGGTTTCGGATAACA	144
	FAK2	102	AGTGAGGAGTGCAAGAGGCAGAT	ATTGGCCAGAACCTTGGCCT	231
	LERL1	93	AGCTTGTGCACTTGTTCTCACAGG	CAGCTGAAGTCGTCATTGCTTCCA	115
	NRG1	85	AACACAAGCTCCCAGAGCAGTAAC	TCTGTATGCCCAGGAAAGGCGTAT	173
	PP2AB	78	TTGGTGTCATGATCGGAATGTGGT	ATAGCAGCCTGGTTCCCACAACGATA	202
	Q7Z4A1	72	AAAGGAGAGAAAGGAGACAGAGCTGG	CAACACCATATCCCACGGCCC	173
	Q7Z2R7	67	TGTAGGTGAATGTGTCCCAAACCTGC	CACCTCTAACAAGACAGTCACACTGAAAC	202
	ZN395	63	TCTGCCATCCTTCCAGATCCCAGT	GCAGCAGCCCAGCTGACACT	144
	ENST332498	60	TTATGAAGTGTTCCCAGTGCCACACT	TTGGCCCAATCTTGTGCTTTCCTC	144
19					
	NP_115613	197	TGGACTGCAGATGCTGAAATCTCTCC	TGACATAGTCCATGGCCAGCTCCA	80
	Q8NB20	181	GCACGCCACGACACTTGACAATTT	CAACTCGCCAGTACCTTATCAAGGAG	134

ACHB3	166	ACGTTCACCACAGATCTTCTTCCAC	TCGAGGACTTTGCCTTTCACTACTGG	80
NP_859074	152	TTTAAACGTGCCGTCTATGTAGC	CGGAATCCTCTATGGAGTCTGTCT	268
ARY1	139	TCACCCTCACCCATAGGAGATTCA	TGTTTGGGCACAAGCTTTCTCTGC	107
NP_689628	127	CTTCCTCCATATCCTCCACAAGAAGC	AAAGGACACCAAATGAAGGAGCGG	107
HAIR	106	ACAGTCAGCGTCACTCAGCACTT	CATAAAGCAGGTGGCAGTCAGGG	161
REEP4	97	AGTGTTGGTCAGATACTGAGGCAG	TTCCTCTTGACCACACGCAGGCT	161
FGF20	89	TTCCGAATGCATCTTTAGGGAGCAG	TGCGGCCAGTGTCTCCATGTTTAT	107
NP_065800	82	GTGGACAACAACACAGCATTGGT	GCTTGTCCATCCGAGCTTTCAA	161
VINEX	76	CCAGAACGAAGACGAGCTGGAG	AAACCAGCCATCGTCACACTGCT	214
NPM2	71	GAGGAAGAGGAAGATGATGAGGATGAGG	ACTTGTTTGACAGGGCTTTGCTCC	161
RN122	67	TCTGGTGAAATGGCTGGAAGTTCG	TGAGGGACTAGCAATGGGCTTGTT	134
CNOT7	63	ACAGGAGGTGGCAGAACAGTTAGA	TCAGATCCTGCCTGATGTTGTGGT	214

20

EGR3	194	GGTGACGTGGAGGCCATGTATC	TGGTGGTAGAGGTTGTAGTCAGGA	144
KI13B	178	CTGACGTGCTGGTGCAGACGAT	ACGCTGTGGGAGAAGTACCCGCTA	115
Q8NH45	163	TGAATCTGGTCACGGTGCTGAGGAA	TGCGACTGCATATCCAGAATCACC	87
NP_116053	149	TACGAAACAAAGACCTCCCTCAGC	TGGCCTCCAGAACTTCTCAGTGAT	87
ACHA2	136	GTTTGCAGTGACCCACATGACCAA	CATCTTGCAGTTCTGCTGGTCGAA	87
RHBT2	124	GTGGTGTTTCCCTACACAAGCAAG	AGAGGCGGTTGGCTAGAATGATGA	87
ARY2	113	GGGTTTACTGTTTGGTGGGCTTCA	CAGCACTTCTTCAACCTCTTCCTC	87
ESCO2	103	CCTAGGGCTTGGCAATGTTCAGAT	GTCGTCTTGCAATGCGCTTTCTTC	87
DOCK5	86	AGCACAAAGGCCAAAGAGTCTCCA	TCAAGGGTGTTGACTGAGGAGGTGAA	87
T2EB	79	TTAGATCAGCATGACCAGCGAGGA	CTTTCTGGGAATTGGGCAGTGCTT	87
CCD25	73	AAAGACCAAAGTCGAGCGGTTCC	CTCATTCCTCTCTTCACGATCTCTGC	115
RBPMS	68	TACCCTCTGTACCCAGCGGAGTTA	AGTGAAGCGGGATAGGTGAAAGCA	346
RBM13	64	ATGGCGACATCTACAACTTCCC	TCTGCCTCCTGTTGTTCCAGGG	144

21

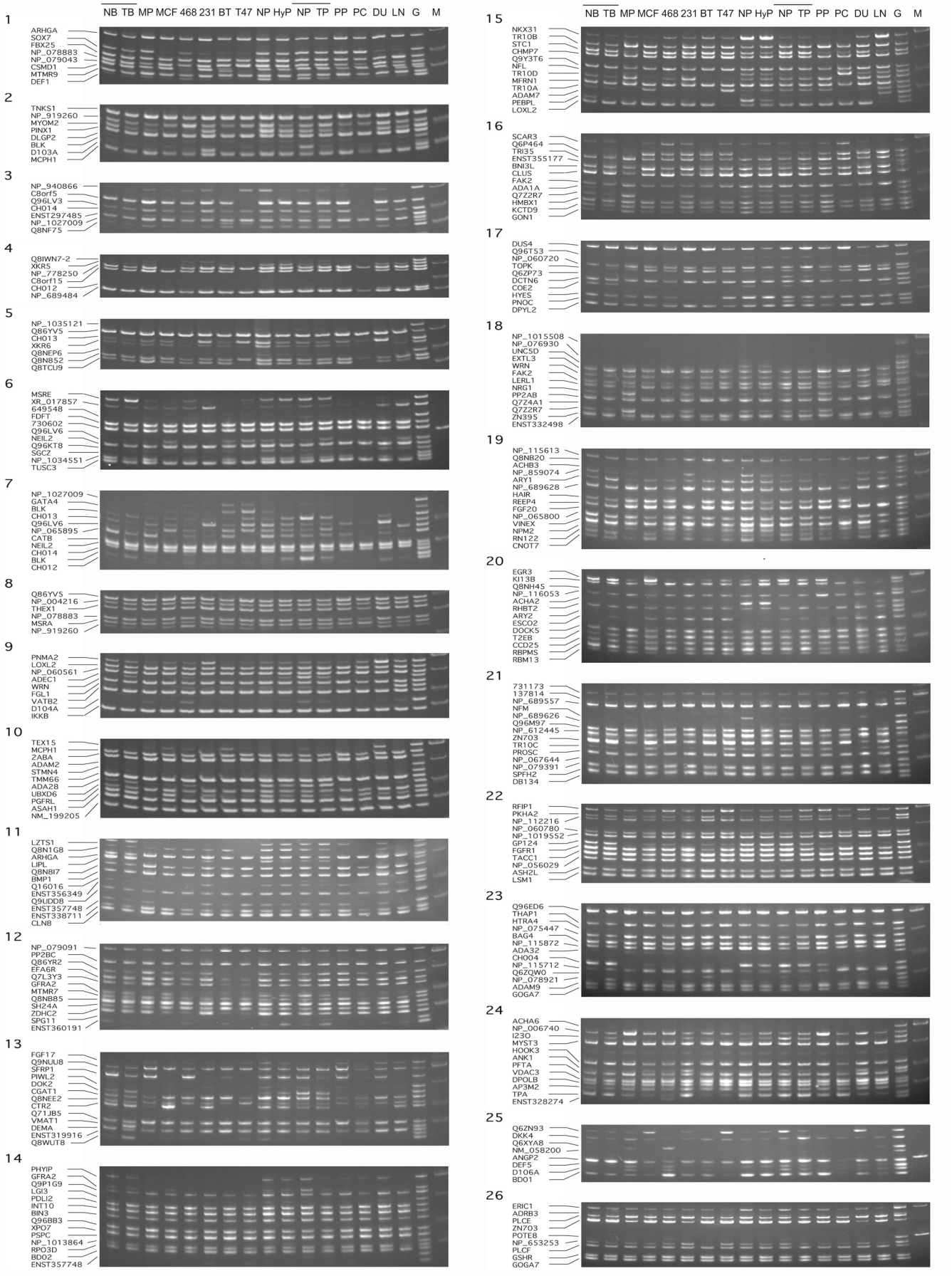
731173	208	AGGTGATGATGACCAAACGGACCA	ACTTTCTTGGCTTCCCATTCAGCC	107
137814	191	CTACAAATGCAAGAGACAGCGCCA	TGTAGCCTCCGTAGCAAGAGTAGG	268
NP_689557	175	AAGCCCAACCATGCAGACATCTTG	CACTGATTGCATAGGCCTGGCTCA	107
NFM	146	CCAGTGAAAGCAACTGCACCTGAA	TCACTAGAGCCTTCCTTCTCGGAT	214
NP_689626	133	ATGCTAGTGGTGGTGGCAGTCAA	GGTTGCAGAGGATGGAGGTGATGA	80
Q96M97	121	GCCACGAGGTGTTTGAGAACTACT	ATGTAGGCAATGAGCACCCAGA	107
NP_612445	110	GAATTATGAGGATATTCAGTGTGCCACC	GGAATACATTTCCAGTTGTTGTCTTAAACG	321
ZN703	100	GACAAGTCCAGCTTCAAGCCCTA	AGGACGAGGAGGAGGTGGAAGACA	321

	TR10C	91	TCCTGCACCATGACCAGAGACACA	TGCTACACTTCCGGCACATCTCT	80
	PROSC	83	ACCATAGCCATCGTGGAGCACATA	TGCCCAAAGCTTCCTATGGTCATC	107
	NP_067644	76	TGGAAGTGGAGCAGAAGATGCTCA	GAGGAGGAATTTCTGCCAATGAGTG	134
	NP_079391	70	TGTGGTCTTACTGAAGGCCCTCTT	CTCAGGTGTAAGGTTTGGATCCCT	107
	SPFH2	65	AAAGCTTCTCATTGCCGCCCAGAA	TCTTCCGCTCTGTCTCTGCTTCCTTT	134
	DB134	61	CTGCAGACTTGAATGCTATGAGAGTG	CTCCAGCTGAAACATACAGTAGGC	214
22					
	RFIP1	192	GCTCAAGCTTGGATAAACAGCTGCC	TGCTGGTGTGGTGAGTGTCAGAAT	68
	PKHA2	176	TTTCAAGTGGGCCCAACTCTATCC	AGGCGTGAACAAGGAGTCCTCTGAA	205
	NP_112216	161	GGATCTACCCACTTTACTGAGGCA	CTAGAATTCCAAACAAGGCTTCAGG	409
	NP_060780	134	CAGTTGGTGGAGCTGGCAAATGA	AATCGGGCAAGGGAACATGAAAGC	136
	NP_1019552	122	TGGTGTGGATCGATTCTGTTTGGG	GCATCAGCCCTCCAGTAATTAGCA	273
	GP124	111	TATCCACGCTGCTCTGGATGGG	TAGGACTGGGAGTAGGCAGAGC	205
	FGFR1	101	TACATGATGATGCGGGACTGCT	TTGGAGGTCAAGGCCACGATGC	205
	TACC1	92	GCCAATGAAGAGATTGCTCAGGTTCG	TCCACCTTCATCTGCTCTTTGCG	205
	NP_056029	84	CAGAAGAGACCTCTGTGGCAGTTA	CATAGTCAATGCGTTGGCCTCCAT	341
	ASH2L	71	TGGTGTCAATCAAGGTGTGGCT	TGTACAGTGAGATGGCTGGGAAGT	341
	LSM1	66	GCTGGAAGCAGAGAAGTTGAAAGTGC	TCTGCTCGAGGAATGGAAAGACCT	273
23					
	Q96ED6	198	TGCCCTCTGCCCTGAACTCTCAT	GTAAGCCGGGTGGAAAGATGATGT	87
	THAP1	167	ATCTTCTGGAGCCACAGGAACAGCTT	TTTCCGCTGGTGCATTGTATCCTC	115
	HTRA4	153	CTCTGGATTGAGAGATCACGATGTA	GTTTCAGGTATGACTGTCAGGAGC	346
	NP_075447	140	CAGTGCAGCTGTTTCCTTCTGTGA	ATTCTGGTGACACAGGAGCCAT	144
	BAG4	128	AATCAGGACCGACTGTACGACCACAA	TTCAGTCATGTAGAGATTGCCGGG	144
	NP_115872	117	CCATGATTGGAATGACATTTGCCT	GCTTCTGTGCAGTGGAAAGTACAAG	288
	ADA32	107	ACACCTGGCTTCTAGGTTTCCTCA	TTCCTCTTCCTTGGCGAACCACTT	144
	CH004	90	ACAAGAAAGCAGAGGAGAGAGCCA	TCTTCTTCAAGGCCATCAGGGCA	173
	NP_115712	83	GATTGACCTGGAGAAGGGCTCACA	TTAGCTGGACAGCTCCAGATGCAA	144
	Q6ZQW0	77	TGAGCTGCCAGTTCCTGAAGGGT	ATAACCCATGGTGAGGAAGCTCAG	144
	NP_078921	72	TCAGACAGATACTGTGGGCTCTGTG	GCCATCATAATGTTGCTGGGCCAT	115
	ADAM9	68	CCACAACCGAAAGTATCATCTCAGGG	TATAAAGGAGGTGCAGGAGCAGGA	231
	GOGA7	64	GAAGAAAGTCTCCAAATACATTCAAGAGC	GAGGAGGCCTTGTGGAGCATAGAT	288
24					
	ACHA6	199	TGATGAGGTGGCCTCTGGACAA	ACCACCCACTGTAATGGCTGATGA	250
	NP_006740	183	CATGACCCATGGCTCTGTGAAATC	TAGTTGCTTTCCTGGGCTGGCTT	375

I23O	168	AGTCAAATCCCTCAGTCCGTGAGT	TCTTATTCTCCTTTGGCTGCTGGC	156
MYST3	154	TTAGCTGGGACTCCTCAAGCACAA	CTGGCATTTGCCCTTGCAATCTCT	156
HOOK3	108	AGACCTTGGTGATTTAAGGCGGCA	GCTGCGTTGGCCTTTCTTAACTCT	94
ANK1	99	TGCAGACGGCTCGATTGTCTCATA	GTACTTGTTCCCTGGAATGAGTGTGG	125
PFTA	91	CGTGGTCTTTCCAAATATCCTAATCTG	CCACAAGAAAGGCAATTAGGTAGGG	313
VDAC3	84	ATTGACTTTATCAGCTTTAATCGATGGG	TAAGCTTCCAGTTCAAATCCCAAGCC	375
DPOLB	78	CTGTTACATCAGGTTGTGGAGCAGT	GAACTTTGTCTCACCCTTTGACAG	313
AP3M2	73	GAGTCTTCAGGCTGGAGCTTCCAAA	CAGCTGCTGGATCTTAAACTGC	188
TPA	69	GCATGACTTTGGTGGGCATCATCA	TGTACACACCCGGGACATCCTTCT	156
ENST328274	65	GTCAATGTGATCCTGGTTATGCTCCTCC	ACTTCCTCCTGGTGATGACATTGC	313
25				
Q6ZN93	179	TTCGCTCTGGGACTCACTCTGAAA	TGTGGTCGTTTGGAGTCCTCCTGAT	94
DKK4	150	TTTGTACTACGATGGAAGATGCAACCC	CCTTCCTGCCTTGTGATTTCTT	188
Q6XYA8	125	CAGTGAGAATTGGATAGCTCCATCAGGG	CCATAGAGCAACTCTGCGAGAGAA	281
NM_058200	114	AGACATGTGAACCACTCAGCCACT	TAAGAGGTCCCGTTTCACTGCGT	188
ANGP2	95	CACCTTAAAGGACTTACAGGGACAGC	CATTTGTCGTTGTCTCCATCCTTTGTGC	141
DEF5	87	AAGAGCTGATGAGGCTACAACCCA	GCAGAGAGTCCATTTCCTGCAAAG	188
D106A	80	AGGGACATGCAAGAACAATTGCGG	TGGTCCGACAGCATTTCAGAGACT	94
BD01	74	AGCAGTGGAGGGCAATGTCTCTAT	TTCCCTCTGTAACAGGTGCCTTGA	281
26				
ERIC1	195	AGAACAAGCAGAATTAGAGAAACAGCAGAG	CTGCTGTCCTCGGGCTGGTACAT	250
ADRB3	179	TGGTCCTGGTGTGGGTCGTGTC	AAGAGGAAGGTAGAAGGAGACGGA	167
PLCE	164	TTATGAGTCACCAGATCCAGAAAGA	TCCAGGTGTTCACATACAGCTTCC	417
ZN703	150	CTATGGCAAGAGCCACTTATCCAC	TGGAGGAAGAGCTGTAGTTACTGG	417
POTE8	95	ACTTGCTGTACGTTGTGGATCAGC	CAGCAGTTTGTCCAAATACATCTTGAG	417
NP_653253	87	TGAGTGTATCAGAACTACAGGCTGC	TGAGCTGTTGTCGCAGTTGTTCCT	167
PLCF	80	GGTGAAGGACACGTTCAAGGAGGA	AGCGGCTCCTGTCCTTGTGGTT	250
GSHR	65	ATGCAGGGACTTGGGTGTGATGAA	GTTGCTCCCATCTTCACTGCAACA	208
GOGA7	61	GAAGATCTATGCTCCACAAGGCCTCC	AACTCGCAGTCCTCGCTCAATA	292

† The gene names are abbreviated. Most names have "_HUMAN" at the end. The "00000" is removed from the numbers of the ENST names and "00" is omitted from the numbers of the NP-genes with 7 digits.

‡ These genes have been delisted from the Ensembl Database in Version 43.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Ensembl Gene ID	External Gene ID	Chr Band	Start Pos (bp)	End Pos (bp)	Set No	SM RT-PCR Results													Illumina DNA Microarray Hybridization Results										
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ENSG00000168204	O95014_HUMAN	p23.3	148350	159420															210	181	156	231	155	161	216	246	199	96	218	255
ENSG00000172748	ZNF596	p23.3	172382	187342															7	-7	-1	9	33	3	-7	8	-10	-13	-2	20
ENSG00000206117		p23.3	222153	223144																									
ENSG00000182366	Q8N852_HUMAN	p23.3	315934	323174	5	396	535	492	216	292	334	481	480	618	83	265	555	805	-29	-13	-15	-6	-11	-32	-17	-24	-24	-16	-12	-15
ENSG00000147364	FBXO25	p23.3	346808	403890	1	581	470	440	441	738	628	483	577	474	361	526	776	497	12	2	18	-2	7	-3	19	37	20	5	15	8
ENSG00000206101	Q8NB26_HUMAN	p23.3	414632	415027															11	-2	2	-6	22	0	-5	80	-5	16	7	-4
ENSG00000180190	NP_778250	p23.3	431647	484974	4	560	789	24	376	848	684	637	668	875	351	702	828	745	84	119	9	47	129	57	45	118	154	31	98	152
ENSG00000180184	Q8NF75_HUMAN	p23.3	554749	571045	3	68	175	60	40	103	259	47	345	113	34	239	60	866												
ENSG00000206100	NP_001013673.1	p23.3	597527	599962															11	0	8	6	15	-6	16	11	-5	2	1	-15
ENSG00000104714	ERIC1	p23.3	604201	671226	26	356	227	191	137	157	175	71	422	243	77	162	53	702												
ENSG00000198010	DLGP2	p23.3	1436941	1637558	2	38	21	16	57	30	56	8	16	50	7	11	59	342												
ENSG00000182372	CLN8	p23.3	1699343	1722145	11	785	1064	957	415	1038	783	1055	959	786	511	790	812	484	173	1214	184	171	153	181	306	166	750	136	260	329
ENSG00000207826	hsa-mir-596	p23.3	1752804	1752880																										
ENSG00000104728	ARHGA	p23.3	1759549	1894206	11	625	884	728	617	904	643	761	546	830	798	1252	1145	361	278	868	302	211	454	111	243	393	606	181	623	287
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ENSG00000176595	KBTBD11	p23.3	1909451	1942509															86	-2	131	23	152	14	25	427	-2	123	284	357
ENSG00000036448	MYOM2	p23.3	1980612	2080779	2	464	127	13	66	481	41	6	474	47	12	524	345	797	192	19	5	31	145	23	20	286	47	46	155	108
ENSG00000183117	CSMD1	p23.2	2762790	3254536	1	38	48	8	5	18	284	45	44	7	4	8	374	409	26	-1	28	6	19	51	28	11	19	3	34	170
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ENSG00000206661	U70	p23.2	4973209	4973342																										
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ENSG00000200621	7SK	p23.2	5755846	5756166																										
ENSG00000147316	MCPH1	p23.1	6251530	6488550	2	459	569	365	368	572	479	484	412	616	206	659	684	698	21	82	14	62	80	29	53	30	38	21	81	46
ENSG00000091879	ANGP2	p23.1	6344580	6408338	25	1805	1346	270	163	570	472	581	1859	1388	81	694	675	1559	122	2	6	3	16	11	17	55	-5	17	7	17
ENSG00000177606	Q96LV3_HUMAN	p23.1	6461593	6462070	3	178	453	208	142	564	151	225	430	537	60	360	446	853												
ENSG00000155189	PLCE	p23.1	6553286	6604592	26	432	873	760	602	781	561	415	569	755	497	675	673	525	245	715	376	279	796	164	211	244	661	200	638	922
ENSG00000186530	XKR5	p23.1	6656129	6680576	4	37	85	20	58	45	67	27	130	26	42	187	191	646												
ENSG00000203559		p23.1	6679710	6683367																										
ENSG00000164825	BD01	p23.1	6715511	6722939	25	466	245	95	1762	328	499	86	1621	1749	67	399	81	1213	273	8	-3	756	30	17	6	207	615	-13	62	10
ENSG00000164822	DEFA6	p23.1	6769625	6771008															-19	-13	-12	-14	-10	-14	-10	-26	-10	-13	-4	-12
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ENSG00000182391		p23.1	6852123	6852287																										
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ENSG00000206042	648637	p23.1	6883503	6884371																										
ENSG00000164816	DEF5	p23.1	6900239	6901669	25	100	265	17	214	57	41	70	480	109	43	84	75	1779	-10	-9	-3	-10	-1	-13	-2	-2	-8	-15	2	2
ENSG00000209978		p23.1	6913533	6913604																										
ENSG00000177306	NP_001035155.1	p23.1	7091658	7093284															3	8	4	11	-6	15	9	-5	16	24	-13	36
ENSG00000206041	645378	p23.1	7099027	7099299																										
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ENSG00000206034	DB109_HUMAN	p23.1	7157776	7164883															13	33	30	20	-3	81	14	42	2	0	29	65
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ENSG00000209992		p23.1	7237354	7237443																										
ENSG00000177257	BD02_HUMAN	p23.1	7259788	7261795	14	12	33	21	36	17	22	15	67	5	10	16	24	202	-15	-5	5	10	-3	15	-5	-5	-7	10	1	5
ENSG00000177243	D103A_HUMAN	p23.1	7273828	7275280															2	9	9	19	691	8	2	-3	6	56	11	16
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ENSG00000206007	645673	p23.1	7630485	7630757																										
ENSG00000206005	FAM90A8	p23.1	7633319	7636880															2	21	8	5	13	23	5	5	9	24	16	32
ENSG00000206003	645673	p23.1	7638132	7638404																										
ENSG00000206002	FAM90A3	p23.1	7640966	7644528															2	21	8	5	13	23	5	5	9	24	16	32
ENSG00000206001	645673	p23.1	7645780	7646052																										
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ENSG00000205999	645673	p23.1	7653426	7653700																										
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ENSG00000186579	D106A_HUMAN	p23.1	7720104	7723985															-14	7	-31	-17	-1	3	2	-14	-8	0	-13	-21
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ENSG00000178287	NM_058200	p23.1	7742812	7758728	25	24	35	29	17	60	42	28	46	36	19	32	29	560	-8	-3	-5	-13	-12	2	-14	-10	-10	-13	-2	-18
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ENSG00000210007		p23.1	7814326	7814415																										
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ENSG00000205989	DB109_HUMAN	p23.1	7885347	7892453															13	33	30	20	-3	81	14	42	2	0	29	65
ENSG00000205987		p23.1	7905105	7905549																										
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ENSG00000205984	NM_001025473.1	p23.1	7928052	7928486																										
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ENSG00000205983	Q6SAD6_HUMAN	p23.1	8083558	8083741																										
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ENSG00000164845	ENST297485	p23.1	8123537	8132145	3	420	928	796	452	631	604	867	715	1016	642	794	793	894												
ENSG00000173295	Q9HBS9_HUMAN	p23.1	8135633	8136076																										
ENSG00000182319	Q86YV5_HUMAN	p23.1	8212676	8276599	5	1233	1298	1439	1249	1274	1023	1559	1118	1414	1035	1212	969	344	1473	1551	1245	1308	744	321	1349	286	1353	622	1216	477
ENSG00000206720	SRP_euk_arch	p23.1	8477272	8477575																										
ENSG00000176305	NP_919260	p23.1	8597319	8598197	2	446	174	325	915	391	224	516	468	202	235	724	395	455												
ENSG00000147324	NP_004216	p23.1	8680942	8787978	8	264	374	373	341	340	336	300	480	484	335	496	547	431	80	34	53	165	74	49	64	125	50	67	149	221
ENSG00000200713	U6	p23.1	8790733	8790846																										
ENSG00000207244	U70	p23.1	8856495	8856628																										
ENSG00000104626	THEX1	p23.1	8897764	8928139	8	364	418	550	472	647	564	580	375	411	331	712	572	456												
ENSG00000210052		p23.1	8953483	8953550																										
ENSG00000129375	Q16016_HUMAN	p23.1	8955447	8956106	11	13	19	21	25	13	21	22	45	17	33	121	21	507												
ENSG00000199207	U7	p23.1	8967375	8967435																										
ENSG00000173281	NP_078883	p23.1	9032916	9045616	1	589	249	493	290	663	614	739	540	284	108	614	319	453	112	65	44	51	75	27	109	54	60	51	93	57
ENSG00000207415	U6	p23.1	9182395	9182498																										
ENSG00000205979	Q8XYA8_HUMAN	p23.1	9261760	9263694	25	19	105	19	523	40	34	49	24	42	56	30	80	1107												
ENSG00000201815	U6	p23.1	9266010	9266109																										
ENSG00000173273	TNKS1	p23.1	9450832	9677266	2	883	976	937	656	945	959	966	736	956	592	959	1003	792	-9	92	32	29	22	31	18	20	27	24	23	37
ENSG00000207701	hsa-mir-597	p23.1	9636592	9636688																										
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ENSG00000210125		p23.1	9826641	9826742																										
ENSG00000175806	MSRA	p23.1	9949188	10323811	8	259	286	271	178	363	236	198	392	331	431	257	226	313	170	125	50	84	192	69	70	104	91	143	96	56
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ENSG00000207128	U6	p23.1	10371143	10371249																										
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ENSG00000171060	NP_001035121.1	p23.1	10567557	10595513	5	42	64	25	46	63	31	46	70	46	50	37	38	469	-2	-10	-13	-1	-9	5	-25	-10	-5	-16	-18	10
ENSG00000210158		p23.1	10581489	10581601																										
ENSG00000171056	SOX7	p23.1	10618688	10625432	1	307	457	52	144	75	43	23	521	619	646	78	34	383	136	234	12	38	15	3	12	102	337	279	18	11
ENSG00000104637	PINX1_HUMAN	p23.1	10659885	10734796	2	552	731	729	638	755	604	765	676	697	704	833	928	697	132	209	118	143	143	61	106	59	141	123	183	249
ENSG00000171044	XKR6	p23.1	10791075	11096258	5	158	151	211	52	163	103	35	84	42	28	84	98	491	37	71	41	61	42	44	50	35	50	61	38	50
ENSG00000207600	hsa-mir-598	p23.1	10930126	10930222																										
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ENSG00000104643	MTMR9	p23.1	11179394	11223080	1	488	659	418	305	596	487	526	458	664	272	533	595	494	184	401	127	92	189	74	132	272	378	58	200	219
ENSG00000164729	AMAC1L1	p23.1	11225853	11227127															19	43	44	12	8	43	17	19	23	14	28	44
ENSG00000154316	Q96LV6	p23.1	11234597	11268371	7	94	92	606	132	44	492	537	136	31	28	68	540	848												
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ENSG00000199368	U6	p23.1	11287330	11287436																										
ENSG00000154319	CH013_HUMAN	p23.1	11316391	11361663	5	122	226	259	52	697	103	759	245	284	92	1097	52	774	143	39	38	13	160	7	166	121	30	26	675	5
ENSG00000184608	CH012_HUMAN	p23.1	11329985	11333461	4	66	27	50	20	48	55	42	97	91	36	261	74	739	40	26	13	17	24	17	49	22	34	27	29	18
ENSG00000136573	BLK	p23.1	11388919	11459522	7	90	72	117	79	50	33	273	1230	56	53	211	45	894	15	-5	-5	17	3	8	-6	-8	5	-3	-12	-7
ENSG00000205925	Q6ZN93_HUMAN	p23.1	11457716	11458195	25	103	67	652	24	111	38	1150	1159	34	23	917	43	898												
ENSG00000170983	CH014_HUMAN	p23.1	11471453	11476259	3	32	87	40	53	46	41	27	93	71	20	78	71	1077	-8	6	-4	-11	-1	-12	-11	-2	-16	12	-1	8

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256~512

128~256

64~128

32~64

16~32

8~16

4~8

2~4

0~2

Ensembl Gene ID	External Gene ID	Chr Band	Start Pos (bp)	End Pos (bp)	Set No	SM RT-PCR Results NB	MP	MCF	468	231	BT	T47	NP	PP	PC	DU	LN	G	Illumina DNA Microarray Hybridization Results NB	MP	MCF	468	231	BT	T47	NP	PP	PC	DU	LN
ENSG00000136574	GATA4	p23.1	11599122	11654920	7	27	81	104	70	41	282	757	28	39	43	39	65	409	-13	-8	-14	-6	-4	4	80	-15	-12	-15	-6	-10
ENSG00000197615	NP_001027009.1	p23.1	11656174	11658141	7	22	51	59	75	38	241	150	66	64	57	56	79	895												
ENSG00000154328	NEIL2	p23.1	11664657	11682263	7	568	554	817	680	928	733	842	879	761	746	926	994	941	302	143	193	262	193	99	177	222	137	151	280	449
ENSG00000177907	730602	p23.1	11684920	11685306	6	933	755	895	1043	1009	965	1005	1028	899	952	1266	965	591												
ENSG00000079459	FDFT1	p23.1	11697573	11734227	6	917	850	1035	1272	1183	1233	1155	1167	989	1081	1447	1211	440	2737	1622	1348	7897	3438	2427	4259	3414	2864	1166	5200	3887
ENSG00000164733	CATB	p23.1	11737442	11763147	7	1537	1518	1697	1822	1743	1671	1696	1267	1656	1132	1545	1483	538	2243	2158	769	1083	1968	328	1521	1383	3477	589	1649	529
ENSG00000210213		p23.1	11808210	11808280																										
ENSG00000177586		p23.1	11823510	11824424																										
ENSG00000210217		p23.1	11830789	11830860																										
ENSG00000205884	DB136_HUMAN	p23.1	11868873	11869517																										
ENSG00000205883	DB135_HUMAN	p23.1	11877239	11879472																										
ENSG00000205882	DB134_HUMAN	p23.1	11888898	11891169	21	31	52	57	50	22	25	29	14	31	42	33	32	297												
ENSG00000182945	NP_958804.1	p23.1	12032086	12033678															-1	2	1	0	3	10	4	-16	4	20	-8	-2
ENSG00000205880		p23.1	12045806	12052951																										
ENSG00000205879	FAM90A2P	p23.1	12067127	12070631															12	45	36	10	11	24	16	5	1	14	63	48
ENSG00000197246	653726	p23.1	12077763	12077915															21	19	23	16	8	30	6	17	28	32	13	31
ENSG00000186523	NP_116305.2	p23.1	12078249	12088988																										
ENSG00000205877		p23.1	12295089	12302245															13	33	30	20	-3	81	14	42	2	0	29	65
ENSG00000205876	389633	p23.1	12316408	12319912															12	45	36	10	11	24	16	5	1	14	63	48
ENSG00000184352	653726	p23.1	12327008	12327160															21	19	23	16	8	30	6	17	28	32	13	31
ENSG00000145002	FAM86B1	p23.1	12327494	12338223																										
ENSG00000205874	Q6SA06_HUMAN	p23.1	12378342	12379002																										
ENSG00000205873	Q6ZP57_HUMAN	p23.1	12382711	12468021																										
ENSG00000197203	Q8NAJ9_HUMAN	p23.1	12480186	12483169															-1	4	-2	-4	-6	4	-9	6	-7	8	-4	-1
ENSG00000177400	Q8NH45_HUMAN	p23.1	12586149	12588894	20	17	36	36	46	36	23	43	20	42	40	26	75	342	3	8	4	11	-6	15	9	-6	16	24	-13	36
ENSG00000210221		p23.1	12614445	12614516																										
ENSG00000154359	NP_689484	p23.1	12623790	12640008	4	852	876	777	440	850	659	707	912	824	234	713	977	717	238	245	174	98	251	94	96	342	248	32	177	510
ENSG00000177357	NP_001034551.1	p22	12853236	12909550	6	481	455	30	11	35	403	13	399	158	37	317	68	460	5	-11	-3	-7	2	7	-4	-11	-7	1	9	2
ENSG00000170941	NP_065895.1	p22	12914144	12928045	7	947	744	56	140	47	519	48	279	211	73	184	65	946	1	-2	-1	2	-16	-18	-16	-8	4	-12	2	-5
ENSG00000206996	U6	p22	12946230	12946336																										
ENSG00000164741	NP_079043	p22	12985243	13416766	1	553	375	480	24	648	124	7	564	242	394	524	384	394	187	120	56	31	162	47	10	50	34	3	26	186
ENSG00000200630	Y	p22	13062058	13062157																										
ENSG00000164743	XR_017857.1	p22	13468723	13470167	6	149	47	94	115	520	26	40	169	31	29	338	595	566	70	46	81	33	113	74	56	31	57	57	102	91
ENSG00000185053	SGCZ	p22	13991744	15140219	6	6	22	14	54	26	22	10	37	47	42	30	18	271	-1	-2	-5	-7	12	-14	-17	-7	-19	-26	-24	-2
ENSG00000199611	U7	p22	14221336	14221396																										
ENSG00000206950	Y	p22	14234435	14234541																										
ENSG00000199127	hsa-mir-383	p22	14755318	14755390																										
ENSG00000104723	TUSC3	p22	15442103	15668529	6	477	471	678	754	702	626	732	610	692	692	769	570	340	509	1232	379	620	804	460	636	1022	1123	339	1091	319
ENSG00000147333	649548	p22	15845005	15845412	6	26	38	21	106	59	69	31	95	22	65	73	42	525												
ENSG00000038945	MSRE	p22	16009761	16094595	6	398	42	32	128	44	56	86	615	23	23	70	40	572	-3	-28	-28	-14	-15	-10	-12	-35	-29	-22	-31	-21
ENSG00000078579	FGF20	p22	16894049	16904061	19	5	22	23	13	29	9	12	24	13	16	15	7	189	-15	-22	-16	-9	-4	-27	-27	-19	-23	-21	-29	-20
ENSG00000155970	NP_859074	p22	16929118	17024524	19	203	40	20	23	389	20	43	81	41	14	96	124	193	14	12	22	23	30	-2	22	31	16	-16	14	11
ENSG00000104219	ZDHC2	p22	17086667	17122305	12	584	633	206	119	851	115	60	651	553	701	863	801	408												
ENSG00000198791	CNOT7	p22	17131108	17148758	19	271	116	335	87	278	172	189	143	168	139	344	490	367	514	403	471	424	550	344	625	452	341	206	841	820
ENSG00000155975	NP_689628	p22	17148851	17197438	19	594	1133	700	497	1525	1015	1056	539	633	240	777	994	292	1	-3	3	-5	9	11	0	9	2	1	-1	-3
ENSG00000003987	MTMR7	p22	17199923	17315207	12	323	446	378	238	320	297	335	295	482	67	266	270	338												
ENSG00000003989	CTR2	p22	17440685	17472296	13	747	69	2550	50	1241	51	41	201	24	75	58	528	483	-6	-13	25	-4	-2	-6	-20	-7	0	-16	-9	-13
ENSG00000104213	PGFRL	p22	17478986	17544896	10	570	29	516	58	243	73	112	312	14	362	178	184	400	1702	2	421	30	107	9	31	154	8	180	84	138
ENSG00000129422	NP_065800	p22	17545584	17702666	19	1039	756	262	625	582	723	507	565	313	81	515	455	175	706	380	89	343	144	200	154	652	324	15	336	282
ENSG00000210287		p22	17649907	17650003																										
ENSG00000104760	FGL1	p22	17766169	17797693	9	61	22	24	66	23	61	25	72	16	18	30	584	424	7	-3	-3	0	-10	-4	-15	2	-21	-16	-3	147
ENSG00000078674	Q8NB85	p22	17824788	17929534	12	992	1249	1152	906	900	1089	1119	1017	965	676	1024	980	310	903	2247	682	548	743	468	723	1203	961	261	1192	899
ENSG00000104763	ASAH1	p22	17958214	17986757	10	695	624	622	648	735	821	780	689	673	551	662	620	449	330	184	276	283	274	259	392	397	231	160	226	325
ENSG00000171428	ARY1	p22	18111882	18273466	19	139	27	128	139	309	371	599	101	46	54	182	206	141	1123	13	99	90	136	137	261	235	26	94	222	198
ENSG00000210291		p22	18164483	18164552																										
ENSG00000156006	ARY2	p22	18293035	18302962	20	17	36	19	50	22	47	14	32	20	29	14	27	410	43	8	-14	11	23	3	16	5	4	12	27	35
ENSG00000181897	Q7L3Y3_HUMAN	p22	18430276	18430590	12	347	645	452	163	460	48	53	330	462	477	579	517	358												
ENSG00000156011	EFA6R	p22	18432351	18915476	12	436	807	608	243	653	112	80	578	557	608	650	588	301	206	403	115	24	268	-7	-15	152	231	155	275	204
ENSG00000187229	Q86YR2_HUMAN	p22	18622695	18636191	12	191	586	212	82	276	23	20	247	457	125	262	140	301												
ENSG00000201157	E2	p22	18881409	18881562																										
ENSG00000104611	SH24A	p21.3	19215408	19298009	12	279	301	617	651	498	980	492	411	421	730	702	579	217	154	75	93	167	115	179	63	93	75	121	199	195
ENSG00000147408	CGAT1_HUMAN	p21.3	19305953	19584552	13	430	112	1100	849	1444	1239	205	1034	96	228	788	374	720	186	0	77	104	143	176	-10	726	-6	23	32	-4
ENSG00000181508	Q8N1G8_HUMAN	p21.3	19500730	19501992	11	104	56	169	202	194	355	18	298	17	51	273	243	330												
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ENSG00000210316		p21.3	19756316	19756810																										
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ENSG00000199211	U6	p21.3	20131570	20131669																										
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ENSG00000199893	5S_rRNA	p21.3	20191965	20192073																										
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ENSG00000210359		p21.3	21945810	21945908																										
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ENSG00000208037	hsa-mir-320	p21.3	22158420	22158501																										
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ENSG00000104635	Q96BB3	p21.3	22280737	22347587	14	716	1220	755	560	796	983	720	484	748	938	981	680	394	380	2118	252	282	568	396	263	259	820	707	1011	592
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ENSG00000147439	BIN3	p21.3	22534186	22558266	14	375	499	452	302	628	380	519	648	421	489	412	210	266	346	397	230	260	259	153	229	275	260	275	245	155
ENSG00000179388	EGR3	p21.3	22601117	22606760	20	1147	133	1381	49	43	118	238	1135	826	34	39	36	481	2	7	66	10	14	38	6	2	18	25	-7	16
ENSG00000134020	PEBPL_HUMAN	p21.3	22626729	22841362	15	150	37	47	49	33	20	10	44	62	26	22	10	167	12	-7	10	-5	13	-6	-4	722	9	2	-1	16
ENSG00000210402		p21.3	22891335	22891585																										
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ENSG00000120889	TR10B	p21.3	22933598	22982637	15	309	888	802	414	824	633	1004	609	1024	492	977	805	236	724	8363	1196	612	2020	343	680	550	6043	1098	1526	1608
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ENSG00000104689	TR10A	p21.3	23104009	23138584	15	85	9	355	24	15	20	742	155	193	13	40	214	173	78	326	97	129	179	45	42	20	180	198	177	128
ENSG00000147457	CHMP7	p21.3	23159705	23175437	15	669	516	714	1046	727	947	1195	754	565	521	1011	711	346	300	172	259	385	324	164	327	374	176	368	439	369
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ENSG00000134013	LOXL2	p21.3	23211501	23281809	9	487	607	516	405	455	415	369	463	592	462	466	549	380												
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ENSG00000200848	U4	p21.2	23513469	23513596																										
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ENSG00000180053	137814	p21.2	23615909	23620056	21	27	16	47	69	17	18	47	48	65	90	80	97	249	13	21	5	21	14	5	16	8	9	39	11	7
ENSG00000207027	U67	p21.2	23719687	23719835																										
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ENSG00000104722	NFM	p21.2	24827188	24832508	21	14	30	14	12	25	33	51	28	16	17	113	9	348	48	51	45	24	35	14	71	69	40	16	69	55
ENSG00000104725	NFL	p21.2	24866240	24869946	15	3	43	11	22	21	10	12	22	14	821	152	261	271	14	14	17	17	10	23	-10	40	24	396	15	43
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ENSG00000104756	KCTD9	p21.2	25341283	25371900	16	228	350	207	201	322	227	239	270	448	254	251	434	641	381	624	287	305	621	261	289	346	552	185	844	362
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ENSG00000120915	HYES	p21.1	27404543	27458403	17	577	213	372	102	60	56	650	706	442	481	463	512	431	329	42	98	47	-7	-5	244	1004	108	156	132	191
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ENSG00000147419	CCD25	p21.1	27646754	27686089	20	250	226	428	222	616	365	655	643	325	183	457	670	175	921	305	353	515	612	214	822	591	418	359	949	227
ENSG00000210467		p21.1	27668478	27668558																										
ENSG00000202242	U6	p21.1	27687081	27687183																										

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- 512~1024
- 256~512
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- 4~8
- 2~4
- 0~2

Ensembl Gene ID	External Gene ID	Chr Band	Start Pos (bp)	End Pos (bp)	Set No	SM RT-PCR Results													Illumina DNA Microarray Hybridization Results											
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ENSG00000211493		p21.1	27873852	27873919																										
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ENSG00000206867	U6	p11.21	41009568	41009674																										
ENSG00000104332	SFRP1	p11.21	41238640	41286149	13	1441	890	21	1328	17	153	29	749	643	35	465	33	394	10839	1453	12	2896	17	245	17	1011	721	60	470	8
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ENSG00000147533	GOGA7	p11.21	41467238	41487650	26	692	582	512	587	580	634	518	605	442	446	473	589	368	907	581	457	433	770	663	909	818	580	261	617	498
ENSG00000147536	NP_115712	p11.21	41505925	41521427	23	72	147	362	472	421	661	455	250	144	739	624	574	391	-9	7	39	120	12	99	71	-26	-14	197	109	105
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ENSG00000165066	NKX6-3	p11.21	41623125	41627168																										
ENSG00000029534	ANK1	p11.21	41629902	41873437	24	52	16	75	24	563	22	34	95	39	11	345	2	449	-11	-27	-22	-13	-10	-21	-8	-1	-17	-20	-12	-29
ENSG00000211539	hsa-mir-486	p11.21	41637116	41637183																										
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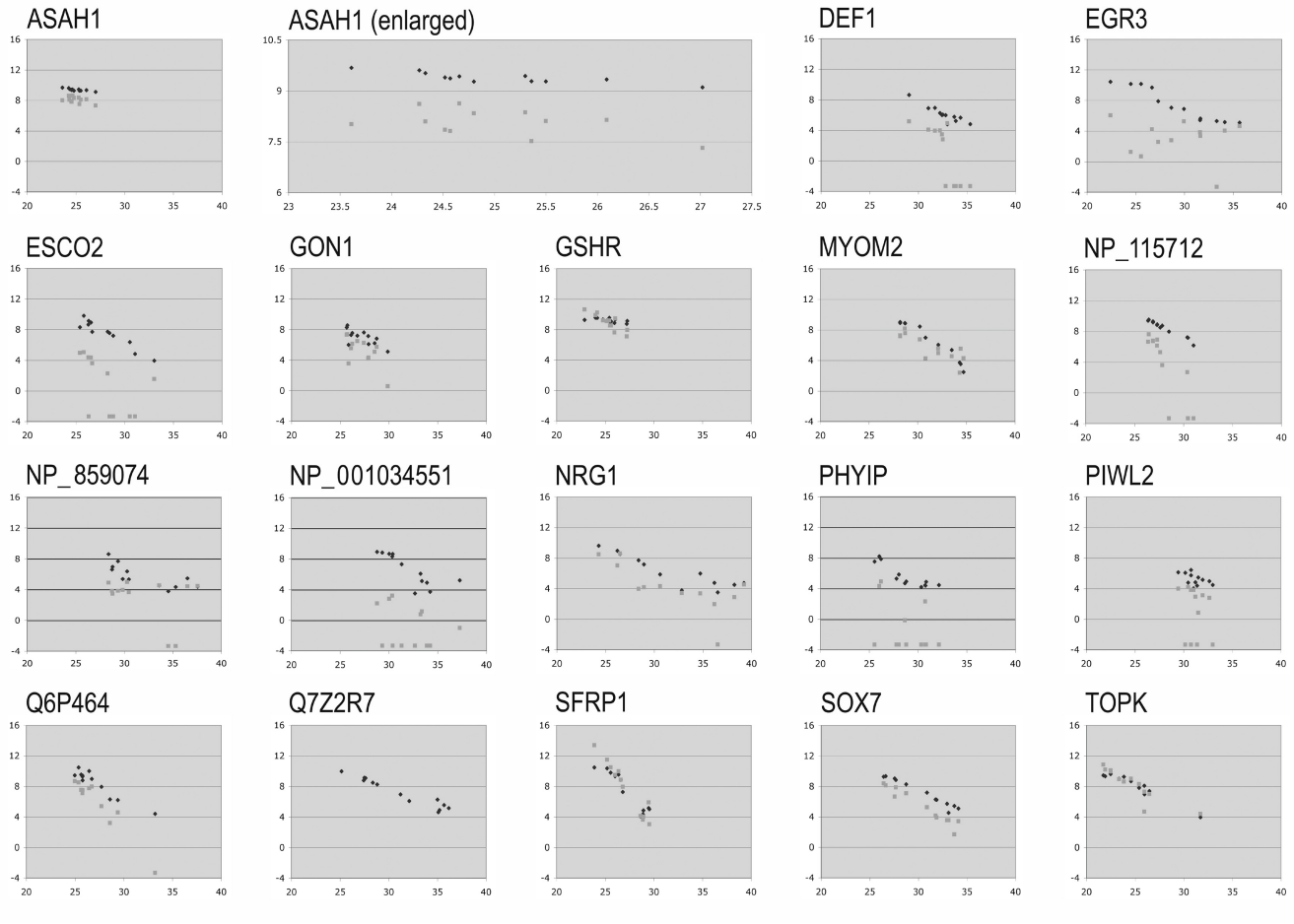
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ASAH1 (enlarged)
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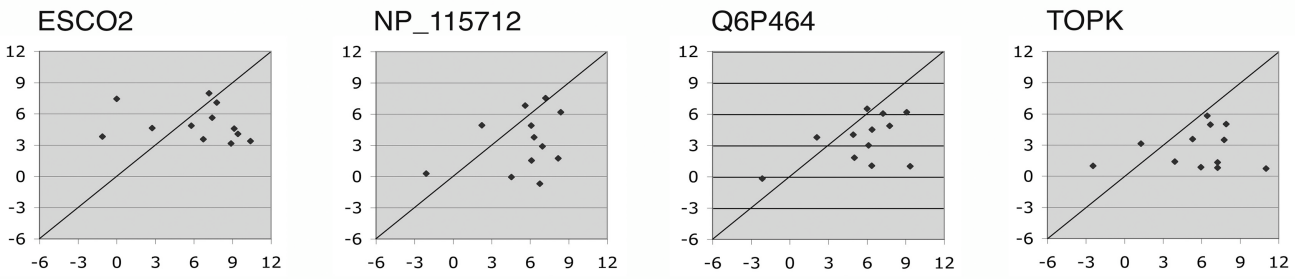
TOPK

Up-regulation Genes



Down-regulation Genes